



Enhancement of lead (Pb) biosorption by Gamma irradiated *Aspergillus japonicus*

Hosam, El-Sayyad^{1,2}; Khaled, F. Elyasergy³; Tahany, M. Abdel Rahaman³; Moustafa, A.S. Aly^{1,4}; Menna Allah, Ashraf^{1*}

¹Middle Eastern Regional Radioisotope Centre for the Arab Countries, Dokki12311, Giza, Egypt; ²Clinical Laboratory Sciences, Al-Ghad International College for Applied Medical Sciences Dammam, Kingdom of Saudi Arabia; ³Microbiology Department, Faculty of Science, Cairo University, Egypt; ⁴Radioisotope Department, Nuclear Research Center, Egyptian, Atomic Energy Authority, Egypt

*Corresponding author E-mail: Menna_Ashraf900@yahoo.com



Received: 30 August, 2020; Accepted: 9 October, 2020; Published online: 12 October, 2020

Abstract

Among the different types of microorganisms; the fungal biomass is considered as an effective mediator for bioremediation of heavy metals, due to its higher surface area and extensive hyphal density in the soil. About seven fungal spp. were isolated from the soil garden of the Middle Eastern Regional Radioisotope Center for Arab Countries (MERRCAC); however, a single isolate of *Aspergillus japonicus* with high potential of metals biosorption was selected for further research during this work. Soil analysis revealed that lead concentration was 5.25 mg/ l. The aims of the present study were to gamma irradiate *A. japonicus* with low doses of 50 to 250Gy, in order to enhance its heavy metal tolerance ability, and to increase its efficacy for removal of lead (Pb) from the soil. Results indicated that combined treatments of *A. japonicus* with lead and gamma irradiation doses displayed more enhancements of its biosorption capacity and gain in dry biomass than single treatment. Treatment with Pb (1950 mg/ l) in combination with gamma irradiation (100 Gy) proved to be optimum for increasing the biosorption capacity of this isolate. However, Pb at 650 mg/ l combined with gamma irradiation at 100 Gy was the optimum for gain in dry biomass. Fourier Transform Infrared Spectroscopic (FTIR) analysis showed that the fungal biomass includes in its surface hydroxyl, carboxyl and amine groups. On the other hand, Transmission electron microscope (TEM) examination of the irradiated *A. japonicus* cells demonstrated accumulation of electron dense Pb on the cell wall and within the fungal cells, this indicate that Pb²⁺ accumulation by fungi occurs by two methods absorption inside the cell and adsorption on the cell wall. The current study expressed a potential new method for enhancing microbial biosorption using low doses of gamma radiation.

Keywords: Fungi, *Aspergillus japonicus*, Gamma radiation, lead, FTIR, TEM

1. Introduction

One of the most serious environmental problems in the last few decades is the pollution of heavy metal in water and soil. [Al-Ghzawi *et al.*, \(2019\)](#) reported that lead (Pb) is a widespread pollutant to the environment and its concentration is increasing dramatically in our ecosystem. Lead concentration is rising rapidly in the environment due to the dramatic increase of world population, and human irresponsible ecological behavior ([Singh *et al.*, 1997](#); [Hadi and Aziz, 2015](#); [Kennedy *et al.*, 2016](#)). Large quantities of Pb were released into the environment through solid and liquid (sludge) wastes, atmospheric emissions (e.g., car exhausts), in addition to human activities including; mining, combustion of fossil fuels, and industrial management practices ([Naja and Volesky, 2009](#)). Lead causes high toxicity and has long-lasting environmental persistence ([Naik *et al.*, 2013](#); [Zeng *et al.*, 2017](#)). Several studies conducted by [Hussain *et al.*, \(2013\)](#); [Shahid *et al.*, \(2014\)](#) confirmed that Pb can cause morphological, physiological, and biochemical disorders in plants. Recently, [Ayangbenro and Babalola, \(2017\)](#) revealed that potential technology needs to be developed to eliminate toxic heavy metal ions found in the polluted environments. Chemical leaching, chemical trapping, electro kinetic remediation, drilling, physical removal, and bioremediation can theoretically be used to remediate heavy metal contamination. However, bioremediation is the best method to remediate heavy metals, as highlighted by [Anastasi *et al.*, \(2013\)](#).

It is necessary to look for novel technologies or new materials for heavy metal removal from wastewater ([Deng *et al.*, 2007](#)). Biosorption is a technology which uses either inactive dead or alive microbial biomass to extract heavy metals from the aqueous solution ([Romera *et al.*, 2007](#)).

An early study conducted by [Selantia *et al.*, \(2004\)](#) documented that algae, yeasts, fungi and bacteria are microorganisms that are used to recover and absorb heavy metals effectively. Later, [Bellion *et al.*, \(2006\)](#) highlighted that fungi have wider surface area and excellent cell wall metal binding properties to absorb such heavy metals. [Prabhu *et al.*, \(2014\)](#) reported that *A. japonicus* var. *aculeatus* is a fungal species that belongs to family *Trichocomaceae* and was isolated from soil. This species of *A. japonicus* is a distinct member of section Nigri of the genus *Aspergillus* ([Al-Musallam, 1980](#); [Gams *et al.*, 1985](#)).

According to [Sreedhar *et al.*, \(2013\)](#), gamma radiation is one of the natural electromagnetic radioactive waves such as X-rays, but is the most powerful form of the electromagnetic radiations, with a very short wavelength. Two ways have been recently used to improve the microbial strain, namely mutation and genetic recombination. One of the most important physical mutagen for fungal strain improvement is the ionizing radiation ([Ramli *et al.*, 2002](#); [Iftikhar *et al.*, 2010](#)). Higher radiation doses are mainly used as an excellent method for food or pharmaceutical sterilization, food preservation and in different food engineering processes ([Dusan, 2004](#); [Hyun-Pa *et al.*, 2006](#)). In contrast to the higher doses of gamma irradiation, low doses of this radiation functions as a growth and a stimulatory factor in fungi ([Cordeiro *et al.*, 1995](#); [Geweely and Nawar, 2006](#); [Dadachova *et al.*, 2007](#); [Robertson *et al.*, 2012](#)). Hence, the objective of the current study was to explore the role of gamma radiation in inducing heavy metal tolerance (in terms of biosorption capacity) in fungi, compared to their un-irradiated counterparts.

2. Material and methods

2.1. Soil sampling

A soil sample was collected from the garden of the Middle Eastern Regional Radioisotope Center for Arab Countries (MERRCAC), Dokki, Egypt.

2.2. Determination of the level of Pb in the soil sample

The Pb content in the soil samples was determined using the wet ashing (oxidation) procedure of [APHA. \(1998\)](#). An aliquot of 0.2 µg (Pb)/ g of soil can be detected using an atomic absorption spectrophotometer, PerkinElmer Model: Analyst 800.

2.3. Isolation and identification of the fungal species

The fungal strain used in this study was isolated from the collected soil samples of the garden of MERRCAC. About 1 g of soil sample was placed in a test tube containing 10 ml of sterile dist. water to make a soil suspension, and tenfold serial dilution was made by transferring one ml of the soil suspension to another test tube containing 9 ml of sterile dist. water. This step was repeated ten times to obtain the dilution of 10^{-10} . An aliquot of 0.1 ml from each of the first three dilutions (10^{-1} , 10^{-2} , and 10^{-3}) was taken and placed on the surface of Potato dextrose agar (PDA) plates, using the spread plate and the pour plate method. The plated were incubated at 30°C for 7 d, and were observed daily to check the presence of filamentous fungi, according to [Wainright *et al.*, \(1993\)](#). Pure cultures of the fungal isolates were obtained by repeated sub-culture on PDA, using the streak-plate dilution technique of [Trivedy and Goel, \(1984\)](#). Cultures were maintained on PDA slants and then kept at 4°C till further use. The recovered fungal isolates were identified at the Regional Center for Mycology and Biotechnology, Al Azhar University, Egypt, according to [Verma *et al.*, \(2008\)](#).

2.4. Source of the Gamma irradiation

Cobalt-60 gamma cell located at the National Center for Radiation Research and Technology,

NCRRT, Egyptian Atomic Energy Authority (EAEA), Nasr City, Egypt, was used for the irradiation treatment. The gamma source gave a dose rate of 1.4 KGy/ h at the time of the assay.

2.5. Preparation of the fungal conidial suspension

Aspergillus japonicus was grown on PDA plate. Conidial suspension was prepared using sterilized water from 7 d old fungal culture under vigorous shaking for 1 min. The conidial density of the suspension was adjusted to 10^7 conidia/ ml, in reference to [Mourya and Jauhri, \(2000\)](#); [Ikram-UI *et al.*, \(2004\)](#).

2.6. Biosorption capacity of the selected fungal spp.

The three selected fungal species were grown in soil samples amended individually with different concentrations of Pb including; 65, 130, 325, 650, 1300, 1950, 2600 and 3250 mg/l of Pb, and then incubated for 7 d, to check their biosorption capacity.

2.7. Estimation of Pb uptake and biosorption capacity of the selected fungal isolate

Irradiation of the fungal conidial suspension was carried out at different doses of the gamma radiation mainly; 50, 100, 150, 200 and 250 Gy. Three concentrations for Pb (i.e. 650, 1300, 1950 mg/ l) were prepared. Sterile PDB was prepared to detect which dose of the radiation stimulated the fungal growth and which one inhibited it. A set of 3 Erlenmeyer flasks were used for each dose. An aliquot of 1 ml of 10^7 irradiated conidia was used to inoculate each set of flasks individually, and then incubated at 30°C for 7 d. The mycelia were isolated from the culture fluid by filtration using Buchner funnel and then washed twice with 10 ml of hot dist. water. Fungal growth was measured gravimetrically after 8 h of drying in an oven at 80°C, cooling it to room temperature, weighting, then re-heated for 1 h, cooled, and weighed again until constant weight was achieved, in reference to [Yonnia *et al.*, \(2004\)](#). The

concentrations of Pb metal in the supernatant before and after inoculation were measured using the Atomic Absorption spectrophotometer (PerkinElmer Model: Analyst 800). The biosorption capacity Q (mg/ g) could be determined by using the following equation of [Fan *et al.*, \(2008\)](#); [Lu *et al.*, \(2020\)](#).

$$q = \{(C_i - C_f) / m\} v$$

where; q (mg/ g) (Biosorption capacity in mg of metal ion uptake per g of fungal biomass).

C_i (mg/ l) is the initial Pb metal concentration

C_f (mg/l) is the final Pb metal concentration

m (g) is the weight of the dry biomass

V (l) is the volume of the PDB medium

2.8. Analysis of the functional groups responsible for metal biosorption using FTIR

2.8.1. Fourier Transform Infra-Red (FTIR)

The functional groups present in the fungal cell wall involved in metal biosorption were detected through FTIR. Samples were prepared following the methods of [Das and Guha, \(2009\)](#); [Xu *et al.*, \(2012\)](#). The used fungal mycelium was lyophilized at -85°C under high vacuum condition using freeze dryer (Virtis EL-65, New York). FTIR spectrum of the samples was recorded using JASCO-6300 FTIR instrument, with a diffuse reflectance mode (DRS8000) attachment.

2.8.2. Transmission electron microscope (TEM)

Ultra-thin sections of the *A. japonicus* mycelia treated with Pb were prepared and mounted on formvar-coated (thermoplastic resins) copper grids for TEM analysis (JeolEm 1400). All assays were performed in triplicates. All mycelial samples were observed in accordance with relevant guidelines and regulations of [Li *et al.*, \(2016b\)](#); [Tian *et al.*, \(2018\)](#).

2.9. Statistical analysis

Data were analyzed statistically by SPSS Statistics (version 25) software, according to Duncan's, (1955) at 5% level.

3. Results and Discussion

3.1. Determination of the Pb level in the soil sample

The soil samples were collected from five random sites at the soil surface and at depths of 20 cm, from the garden of MERRCAC. Soil analysis revealed that Pb concentration was 5.25 mg/ l. Elevated Pb in this soil of MERRCAC may be attributed to the location of the Radioisotope Center to the Giza traffic department.

3.2. Isolation and identification of the soil fungi

After isolation and identification, about seven fungal species belonging to five different genera were recovered; however, the most abundant three fungal isolates were identified as; *A. japonicus*; *Rhizopus oryzae* and *A. flavus*. These species are known to have the potential to absorb the different heavy metals (i.e. Pb), thus were selected for this study to test for bioaccumulation of Pb.

3.3. Biosorption capacity of the selected fungal spp.

Results demonstrated in Fig. (1) indicated that the biosorption capacity of *R. oryzae* tolerates Pb (II) up to 650 g/ l, followed by *A. flavus* which showed maximum biosorption capacity at 1300 mg/ l; however, *A. japonicus* expressed the maximum biosorption capacity of 1950 mg/ l.

Thus, *R. oryzae* is observed to be more sensitive, followed by *A. flavus*, whereas, *A. japonicus* is the most tolerant to Pb concentration. This prompted further investigation on this isolate, to study its lead biosorption on enhancement using gamma radiation effect.

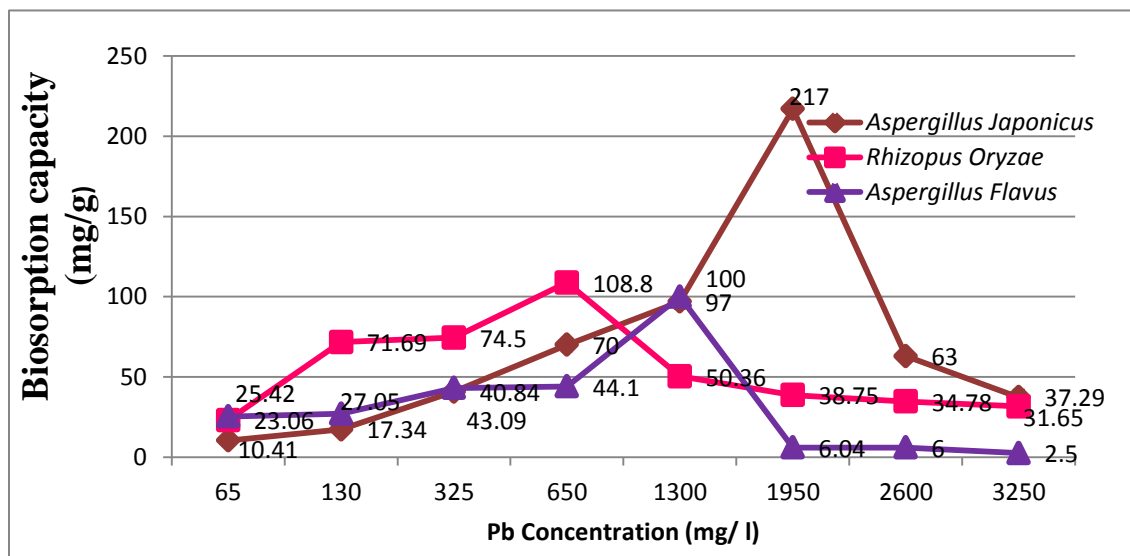


Fig. 1: Biosorption capacity of selected fungal species (*A. japonicus*, *R. oryzae* and *A. flavus*)

3.4. Estimation of the biosorption capacity and dry biomass of the selected *A. japonicus*

The effect of gamma radiation on the dry biomass of *A. japonicus* when grown on different concentrations of lead (0.0, 650, 1300 and 1950 mg/l) is shown in Fig. (2). The fungal biomass decreased with increasing the Pb concentration, compared to the control. Similarly, [Al-Kadeeb and Siham \(2007\)](#) reported that the mycelial growth of *A. niger* was not affected by the low concentration of Pb; and high concentrations caused little inhibition on its growth. Conversely, *A. ochraceus*, *A. parasiticus* growth was inhibited at high concentrations of metals. On the other hand, gamma irradiation induced significant increase in the dry biomass of *A. japonicus*. Fig. (2) shows that *A. japonicus* exposed to a dose of 100Gy of gamma rays, resulted in an increase in its dry biomass, compared to the un-irradiated control. Interestingly, *A. japonicus* exposed to a dose of 100Gy exhibited 1.3, 1.2 and 1.47 times more gain

in the dry biomass when grown in 650 mg/l, 1300 mg/l and 1950 mg/l; respectively, compared to un-irradiated control. This may be attributed to an increase in Pb removal efficiency of *A. japonicus* as a result of gamma irradiation treatment. Similar to the current results, a study conducted by [Das et al., \(2016\)](#) revealed that exposure of *A. terreus* to gamma radiation empowered it to withstand 1.13 times more Zn, thus represented higher growth under Zn stress, in addition to an improved efficiency of Zn removal than the un-irradiated strain. Moreover, an increase in heavy metals (Zn, Cd, Pb) uptake by several fungi including; *A. niger*, *A. terreus*, and *Penicillium cyclopium*, was recognized after subjecting these strains to gamma radiation during the study of [Das et al., \(2017\)](#). In accordance with the current results, the recent study of [Das et al., \(2019\)](#) recorded that exposure of *A. niger* and *P. cyclopium* to gamma radiation led to an increased growth yield and more efficient Pb uptake, compared to the un-exposed control fungi.

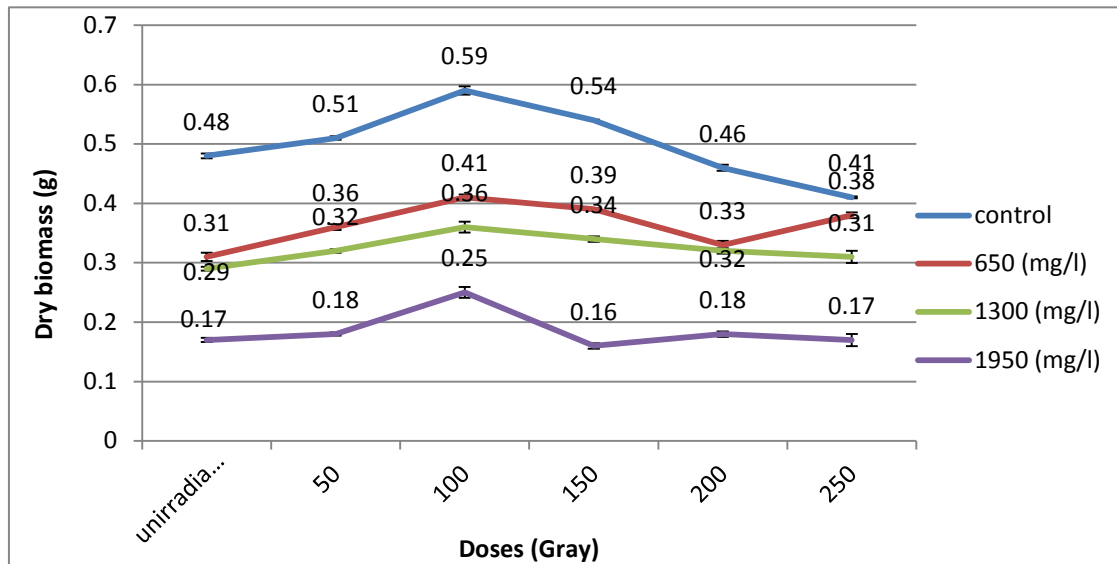


Fig. 2: Dry biomass of *A. japonicus* with and without exposure to gamma irradiation, grown in Pb-supplemented media. Where; Error bars represent standard deviation for $n = 3$. $P \leq 0.05$ was considered significant

Results presented in Fig. (3) illustrate that Pb (II) at 650 mg/ l (the least concentration of Pb used) combined with gamma irradiation at 100 Gy are the optimum treatments for increasing the biosorption capacity, where it manifested 1.3 fold more in the biosorption capacity compared to the untreated counterparts. However, when *A. japonicus* was grown on media containing higher concentrations of Pb (II) (1300 and 1950 mg/ l), an increase of 1.25 and 1.2 folds; respectively, were recorded compared to the respective un-irradiated control (Fig. 2). On the other hand, treatment with irradiation doses of 200 and 250 Gy exhibited a decline in the biosorption capacity, compared to the exposure to an irradiation dose of 100 Gy.

A previous study conducted by [Ziombra, \(1994\)](#) indicated that *Pleurotus* sp. spore germination could be increased by treatment with gamma radiation doses of 100 and 200 Gy. The work of [Geweely and Nawar, \(2006\)](#), revealed that subjecting *A. tenuissima* and *Stemphylium botryosum* to gamma

irradiation dose of 250 Gy resulted into the highest germination rate and growth stimulation.

3.5. Detection of the fungal functional groups using FTIR

In the current study, the use of FTIR spectroscopy enabled the detection of changes occurring in the different functional groups of *A. japonicus* biomass before and after irradiation treatments by comparing the peaks in their spectra, represented in Table (1) and Fig. (4).

FTIR spectra of the different experimental treatments of *A. japonicus*, showed that a peak around 3356 cm^{-1} region is attributed to the bonded OH stretching vibration as well as N-H stretching ([Damodaran *et al.*, 2013](#)). The stretching vibration of OH group in the loaded biomass moved from 3356 cm^{-1} in control group (A) to 3370 cm^{-1} and to 3300 cm^{-1} in the case of Pb loaded biomass (B) and the loaded biomass after exposure to 100 Gy gamma irradiation (C), respectively.

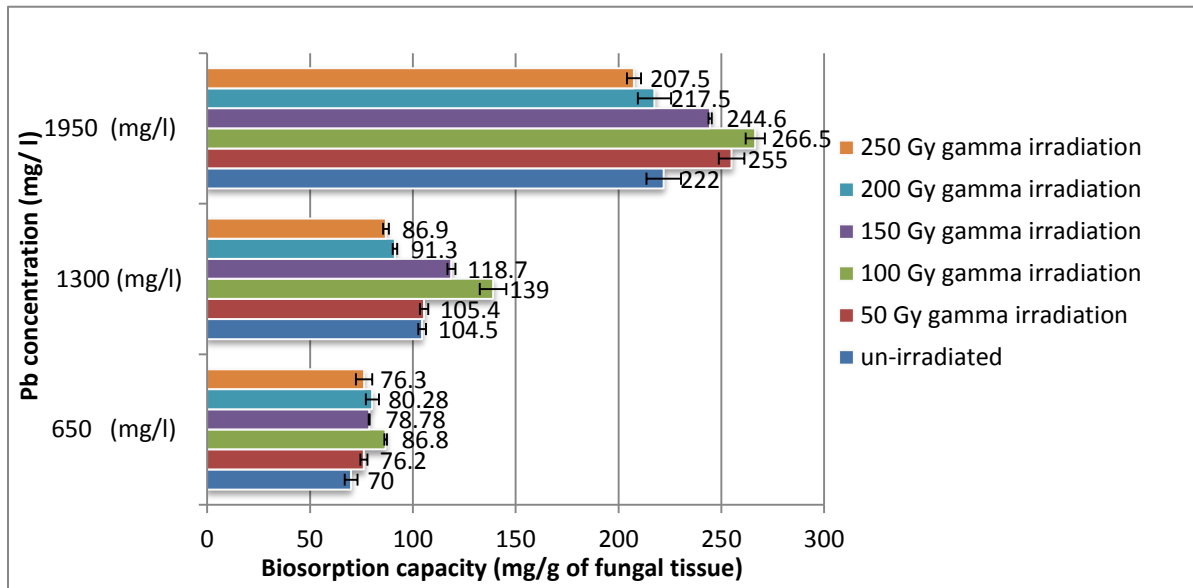


Fig. 3: Biosorption capacity of Pb by *A. japonicus* with or without exposure to gamma irradiation. Where; Error bars represent standard deviation for n = 3. P ≤ 0.05 was considered significant

Table 1: Detection of the surface functional groups of the *A. japonicus* fungal biomass before and after biosorption using FTIR spectral analysis

Before biosorption (A) (cm ⁻¹)	After biosorption of Pb (B) (cm ⁻¹)	After exposure to 100 Gy gamma radiation in combination with Pb (C) (cm ⁻¹)	Functional groups
3356	3370	3300	O-H stretching; N-H stretching
2925	2925	2928	C-H stretching
1746	1745	Disappeared	C=C stretching in carboxyl or C=N amide groups
1620	1618	1654	C=C stretching or C=N amide stretching
1417	1418	1418	C-N deformation, C-O-H stretching
1080	1077	1076	CO stretching of alcohols and carboxylic acids
888	887	852	C-H deformation, N-H vibration

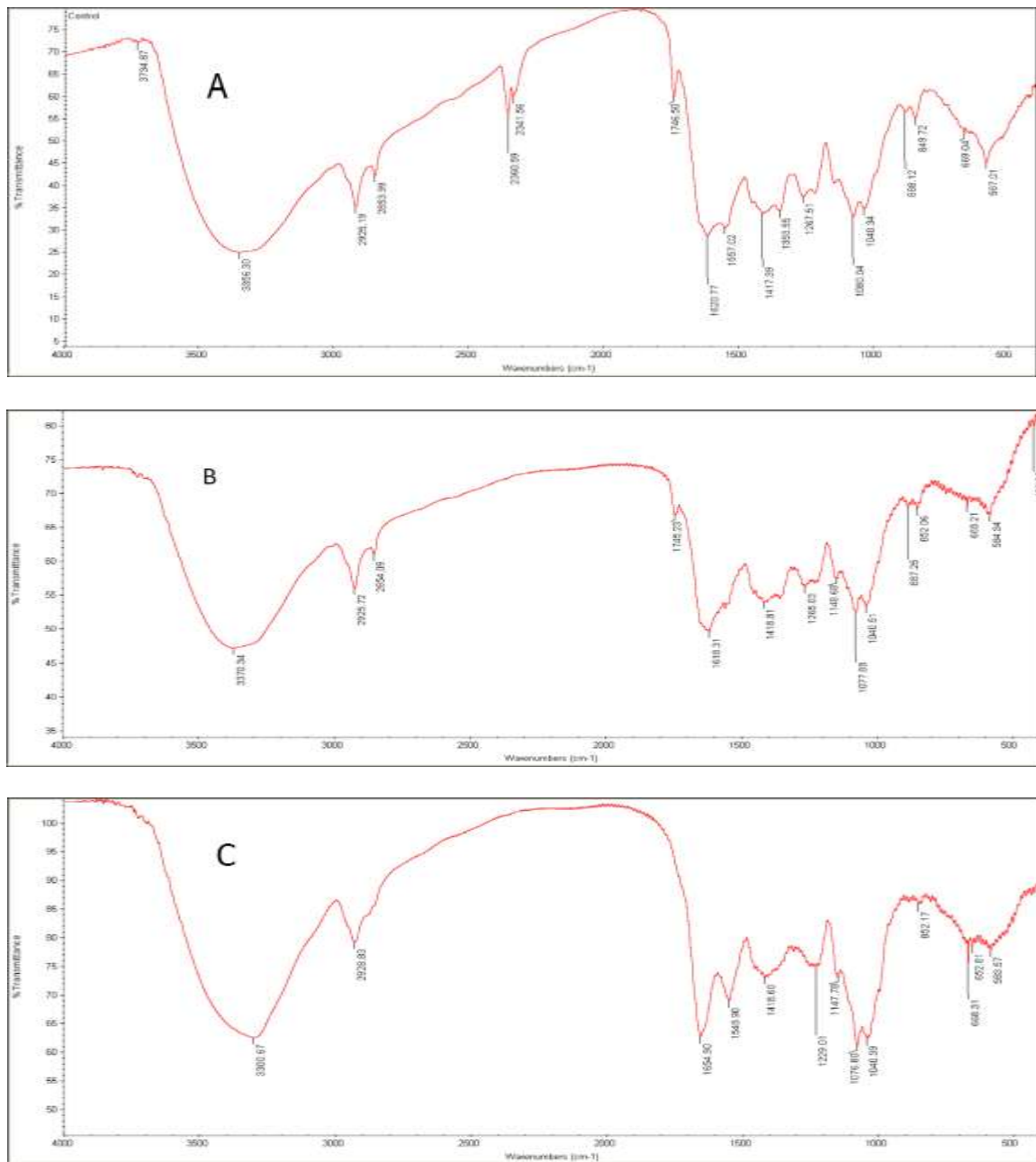


Fig. 4. FTIR spectra of *A. japonicus* mycelia; (A) Untreated control, (B) After treatment with Pb (1950 mg/ l), (C) After exposure to 100 Gy gamma radiation in combination with 1950 mg/ l of Pb supplemented in an enriched media

These findings showed that on the biomass surface there were chemical interactions between the metal ions and the hydroxyl group. The peak observed for the unloaded biomass at 1620 cm^{-1} (A) region that was attributed to C=O stretching vibration and NH deformation (amide I) ([Xu *et al.*, 2009](#)), is shifted to 1618 cm^{-1} in the Pb loaded biomass (B), and to 1654 cm^{-1} in the Pb loaded biomass in addition to 100 Gy gamma radiation (C). This data confirmed that the interactions with carbonyl functional group are present between the biomass and the Pb ions.

The peak that corresponds to reflect the C-O, C-N stretching vibration groups ([Chen *et al.*, 2019](#)) at 1080 cm^{-1} in the biomass of the control group (A) is shifted to 1077 cm^{-1} in Pb loaded biomass (B), and to 1076 cm^{-1} in Pb loaded biomass in addition to gamma radiation (C). These results point out that the listed functional groups (Hydroxyl, Carboxyl) were mainly involved in the biosorption of Pb on the biomass of *A. japonicus*. Moreover, after biosorption of metal ions, no change in the frequency is observed in the biomass group N-H. This implies that this group did not take part in the biosorption of metal ions. Also a prominent variation was revealed when two peaks of 2853 cm^{-1} and 1746 cm^{-1} that were attributed to the symmetric vibration of CH₂ and C=O stretching in the carboxyl groups; respectively, had disappeared from group (C) only, indicating further stress ([Simonescu and Ferdeş, 2012](#)). Thus, it could be concluded that gamma irradiation, being an external stress factor; induced some metabolic changes in the exposed fungal strain involving the main functional groups. Consequently, *A. japonicus* have hydroxyl, carboxyl and amine groups on its surface. Our results are in agreement with [Akar *et al.*, \(2005\)](#), who found that similar FTIR results were reported for the biosorption of Pb (II), Cd (II) and Cu (II) onto *Botrytis cinerea* fungal biomass, and with the results of [Anayurt *et al.*, \(2009\)](#) who revealed that the same FTIR results were reported for the biosorption of Pb (II) and Cd (II) from aqueous

solution of the macrofungus (*Lactarius scrobiculatus*) biomass. Other recent investigators who studied the adsorption, bioaccumulation and bioleaching occurring after treatment with distinct heavy metals including; Pb, Ni, Cr, Zn, also used the FTIR spectrum analysis, to determine the variations occurring in the functional groups in different fungi ([Aytar *et al.*, 2014](#); [Qayyum *et al.*, 2016](#); [Feng *et al.*, 2018](#); [Xu *et al.*, 2020](#)).

3.5. Observation of the Pb treated mycelial cells of *A. japonicus* using TEM

Alteration of the usual cylindrical shape and hyphal structure of the Pb-exposed *A. japonicus* is observed under TEM. In the present study, TEM demonstrated electron dense accumulation of Pb precipitates on the fungal cell surface and cell wall, as well as electron dense granules within the cells. This indicate that Pb²⁺ accumulation by fungi occurs by two methods; absorption inside the cell and adsorption on the cell wall. During the recent study of [Tian *et al.*, \(2019\)](#), the TEM images showed that Pb cations progressively reached the cell wall of *A. niger* and *P. oxalicum* as the concentrations of Pb increased from 500 to 1500 mg/l.

Conclusion

The data of obtained from the current study disclosed the potential of 100 Gy gamma radiation in modulating Pb (II) removal by *A. japonicus*. The gamma irradiated *A. japonicus* by 100 Gy exhibited an increased growth, as well as an enhanced ability to adsorb and retain Pb II. This was achieved even when Pb stress reached a high concentration up to 1950 mg/l. FTIR studies revealed the role of various functional groups such as; hydroxyl, carboxyl and amine groups in biosorption of Pb, as well as the mechanism of ion exchange. On the other hand, TEM detected electron dense accumulates of Pb on the fungal cell surface as well as inside the cell. Both of FTIR and TEM clearly indicated that Pb is accumulated in fungi by absorption and adsorption.

Findings of the current study expressed a novel step of microbial biosorption using a cost-effective approach, for possible bioremediation of Pb by *A. japonicus*.

Acknowledgment

The authors are grateful to the Middle Eastern Regional Radioisotope Centre for the Arab Countries who supported this work financially, and also thank all members of the Middle Eastern Regional Radioisotope Centre for the Arab Countries for providing the necessary facilities, and for allowing the use of the different laboratories.

Conflict of interest

There is no any conflict of interests between the authors of this study.

Funding source

This work was funded by the Middle Eastern Regional Radioisotope Center for Arab Countries, Dokki12311, Giza, Egypt.

Ethical Approval

Non-applicable.

4. References

Akar, T.; Tunali, S. and Kiran, I. (2005). *Botrytis cinerea* as a new fungal biosorbent for removal of Pb (II) from aqueous solutions. *Biochemical Engineering Journal*. 25(30): 227-235.

Al-Ghzawi, A.L.A.; Al Khateeb, W.; Rjoub, A.; Al-Tawaha, A.R.M.; Musallam, I. and Al Sane, K.O. (2019). Lead toxicity affects growth and biochemical content in various genotypes of barley (*Hordeum vulgare* L.). *Bulgarian Journal of Agricultural Science*. 25(1): 55-61.

Al-Kadeeb, A. and Siham, (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: 1019-1024.

Al-Musallam, A. (1980). Revision of the black *Aspergillus* species. Ph.D. Thesis, State University Utrecht, The Netherlands.

Anastasi, A.; Tigini, V. and Varese, G.C. (2013). The bioremediation potential of different ecophysiological groups of fungi. In: M.G., Ebrahim; R.D. Younes and V. Ajit (Eds.), *Fungi as Bioremediators*. New York, NY: Springer, pp. 29-49.

Anayurt, R.A.; Sari, A. and Tuzen, M. (2009). Equilibrium, thermodynamic and kinetic studies on biosorption of Pb (II) and Cd (II) from aqueous solution by macrofungus (*Lactarius scrobiculatus*) biomass. *Chemical Engineering Journal*. 151: 255-261.

APHA. (1998). Standard methods for the examination of water and wastewater, 20th Edition. American Public Health Association, Washington, D.C.

Ayangbenro, A.S. and Babalola, O.O. (2017). A new strategy for Heavy Metal Polluted Environments: A Review of Microbial Biosorbents. *International Journal of Environmental Research and Public Health* .14(1): 94.

Aytar, P.; Gedikli, S.; Buruk, Y.; Cabuk, A. and Burnak, N. (2014). Lead and nickel biosorption with a fungal biomass isolated from metal mine drainage: Box–Behnken experimental design. *International Journal of Environmental Science and Technology*. 11: 1631-1640.

Bellion, M.; Courbot, M.; Jacob, C.; Blaudez, D. and Chalot, M. (2006). Extracellular and cellular mechanisms sustaining metal tolerance in ectomycorrhizal fungi. *FEMS Microbiology Letters*. 254(2): 173-181.

Chen, S.H.; Cheow, Y.L.; Ng, S.L. and Ting, A.S.Y. (2019). Mechanisms for metal removal

established via electron microscopy and spectroscopy: a case study on metal tolerant fungi *Penicillium simplicissimum*. Journal of Hazardous Materials. 362: 394-402.

Cordeiro, V.K.; Luna, E.A. and Azevedo, J.L. (1995). Survival and mutant production induced by mutagenic agents in *Metarhizium anisopliae*. Science Agriculture. 52: 548-554.

Dadachova, E.; Bryan, R.A.; Huang, X.; Moadel, T.; Schweitzer, A.D.; Aisen, P.; Nosanchuk, J.D.; and Casadevall, A. (2007). Ionizing radiation changes the electronic properties of melanin and enhances the growth of melanized fungi. Public Library of Science One. 2: e457.

Damodaran, D.; Balakrishnan, R.M. and Shetty, V.K. (2013). The uptake mechanism of Cd (II), Cr (VI), Cu (II), Pb (II), and Zn (II) by mycelia and fruiting bodies of *Galerina vittiformis*. BioMed Research International. 2013:1-11

Das, D.; Chakraborty, A. and Santra, S.C. (2019). Assessment of Lead tolerance in gamma exposed *Aspergillus niger* Van Tieghem and *Penicillium cyclopium* Westling. International Journal of Radiation Biology. 95(6): 771-780.

Das, D.; Chakraborty, A. and Santra, S.C. (2017). Heavy metal removal efficacies by gamma absorbed fungal strains on optimization of pH and temperature. Academia Journal of Biotechnology. 5(4): 051-056.

Das, D.; Chakraborty, A. and Santra, S.C. (2016). Effect of Gamma Radiation on Zinc Tolerance Efficiency of *Aspergillus terreus* Thorn. Current Microbiology. 72: 248-258.

Das, S.K. and Guha, A.K. (2009). Biosorption of hexavalent chromium by *Termitomyces clypeatus* biomass: kinetics and transmission electron microscopic study. Journal of Hazardous Materials. 167: 685-691

Deng, Z.Y.; Zhang, J.W.; Wu, G.Y.; Yin, Y.L.; Ruan, Z.; Li, T.J.; Chu, W.Y.; Kong, X.F.; Zhang, Y.M.; Fan, Y.W.; Liu, R. and Huang, R.L. (2007). Dietary supplementation with polysaccharides from *Semen cassiae* enhances immunoglobulin production and interleukin gene expression in early-weaned piglets. Journal of the Science of Food and Agricultural. 87: 1868-1873.

Dusan, R. (2004). Twenty years of radiation sterilization in Croatia. Radiation Physics and Chemistry. 71: 597-602.

Fan, T.; Liu, Y.; Feng, B.; Zeng, G.; Yang, C.; Zhou, M.; Zhou, H.; Tan, Z. and Wang, X. (2008). Biosorption of cadmium (II), zinc (II) and lead (II) by *Penicillium simplicissimum*: Isotherms, Kinetics and thermodynamics. Journal of Hazardous Materials. 160: 655-661.

Feng, C.I.; Li, J.; Li, X.; Li, K.I.; Luo, K.; Liao, X.S. et al. (2018). Characterization and mechanism of lead and zinc biosorption by growing *Verticillium insectorum* J3. Public Library of Science ONE. 13(12): e0203859.

Gams, W.; Christensen, M.; Onions, A.H.S.; Pitt, J.I. and Samson, R.A. (1985). Infrageneric taxa of *Aspergillus*. In: R.A. Samson & J.I. Pitt (Eds.), Advances in *Penicillium* and *Aspergillus* Systematics. Plenum Press, New York, pp. 55-62.

Geweely, S.I.N. and Nawar, L.S. (2006). Sensitivity to gamma irradiation of postharvest pathogens of pear. International Journal of Agricultural and Biological Engineering 8(6): 710-716.

Hadi, F. and Aziz, T. (2015). A mini review on lead (Pb) toxicity in plants. Journal of Biology and Life Science. 6(2): 91-101.

Hussain, A.; Abbas, N.; Arshad, F.; Akram, M.; Khan, Z.I.; Ahmad, K. and Mirzaei, F. (2013). Effects of diverse doses of Lead (Pb) on different

growth attributes of *Zea mays* L. Agricultural Sciences. 4(5): 262-265

Hyun-Pa, S.; Dong-Ho, K.; Cheorun, J.; Cheol-Ho, L.; Kyong-Soo, K. and Myung-Woo, B. (2006). Effect of gamma irradiation on the microbiological quality and antioxidant activity of fresh vegetable juice. Food Microbiology. 23: 372-378.

Iftikhar, T.; Niaz1, M.; Hussain, Y.; Abbas, S.Q.; Ashraf, I. and Zia, M.A. (2010). Improvement of selected strains through gamma irradiation for enhanced lipolytic potential Pakistan journal of Botany. 42(4): 2257-2267.

Ikram-UI, H.; Sikander, A.; Qadeer, M.A. and Javed, I. (2004). Citric acid production by selected mutants of *Aspergillus niger* from cane molasses. Bioresource Technology. 93: 125-130.

Kennedy, C.; Yard, E.; Dignam, T.; Buchanan, S.; Condon, S.; Brown, M.J.; Raymond, J.; Rogers, H.S.; Sarisky, J.; Castro, R.D.; Ileana Arias, I.; Patrick, N. and Breyse, P.N. (2016). Blood lead levels among children aged < 6 years - Flint, Michigan, Morbidity and Mortality Weekly Report (MMWR). 25: 650-654.

Li, Z.; Wang, F.W.; Bai, T.S.; Tao, J.J.; Guo, J.Y.; Yang, M.Y. et al. (2016b). Lead immobilization by geological fluorapatite and fungus *Aspergillus niger*. Journal of Hazardous Materials. 320: 386-392.

Lu, N.; Hua, T.; Zhaia, Y.; Qina, H.; Aliyevaa, J. and Zhanga, H. (2020). Fungal cell with artificial metal container for heavy metals biosorption: Equilibrium, kinetics study and mechanisms analysis. Environmental Research. 182: 109061.

Mourya, S. and Jauhri, K.S. (2000). Production of citric acid from starch hydrolysate by *Aspergillus niger*. Microbiology Research. 155: 37-44.

Naik, M.M.; Khanolkar, D. and Dubey, S.K. (2013). Lead resistant *Providencia alcalifaciens*

strain 2EA bioprecipitates Pb⁺² as lead phosphate. Letters in Applied Microbiology. 56: 99-104.

Naja, G.M. and Volesky, B. (2009). Toxicity and sources of Pb, Cd, Hg, Cr, As, and radionuclides in the environment. Heavy metals in the environment. CRC Press. pp. 13-62.

Prabhu, R.; Pai, K.M.; Prabhu, G. and Shrilatha. (2014). A lecture in medical physiology: PowerPoint versus chalkboard. South East Asian Journal of Medical Education. 8(1): 72-76.

Qayyum, S.; Khan, I.; Bhatti, Z.A.; Tang, F. and Peng, C. (2016). Fungal strain *Aspergillus flavus* F3 as a potential candidate for the removal of lead (II) and chromium (VI) from contaminated soil. Main Group Metal Chemistry. 39(3-4): 93-104.

Ramli, A.A.M.; Besar, I.; Khalid, M.A. and Hassan, S. (2002). Biological effects of ionising radiation. In: Radiation Safety, 2nd edition. Malaysian Institute for Nuclear Technology Research. Malaysia. pp. 27-28.

Robertson, K.L.; Mostaghim, A.; Cuomo, C.A.; Soto, C.M.; Lebedev, N.; Bailey, R.F. and Wang, Z. (2012). Adaptation of the Black Yeast *Wangiella dermatitidis* to ionizing radiation: molecular and cellular mechanisms. Public Library of Science One. 7: e48674

Romera, E.; Gonzalez, F.; Ballester, A.; Blazquez, M.L. and Munoz, J.A. (2007). Comparative Study of Bio-sorption of Heavy Metals Using Different Types of Algae, Bioresource Technology. 98: 3344-3353.

Selantia, A.; Bakhti, M.Z.; Madani, A.; Kertous, L. and Mansouri, Y. (2004). Biosorption of Cd²⁺ from Aqueous Solution by a NaOH-treated Bacterial Dead *Streptomyces rimosus* Biomass. Hydrometallurgy. 75: 11-24.

Shahid, M.; Pourrut, B.; Dumat, C.; Nadeem, M.; Aslam, M. and Pinelli, E. (2014). Heavy-metal-induced reactive oxygen species: phytotoxicity and

physicochemical changes in plants. In *Reviews of Environmental Contamination and Toxicology*. 232: 1-44.

Simonescu, C.M. and Ferdeş, M. (2012). Fungal biomass for Cu (II) uptake from Aqueous system. *Polish journal of Environmental Studies*. 21(6), 1831-1839.

Singh, R.P.; Tripathi, R.D.; Sinha, S.K.; Maheshwari, R., and Srivastava, H.S. (1997). Response of higher plants to lead contaminated environment. *Chemosphere*. 34(11): 2467-2493.

Sreedhar, M.; Chaturvedi, A.; Aparna, M.; Pavan, K.D.; Singhal, R.K. and Venu-Babu, P. (2013). Influence of gamma radiation stress on scavenging enzyme activity an cell ultra-structure in groundnut (*Arachis hypogaea* L.). *Advances in Applied Science* .4(2): 35-44.

Tian, D.; Jiang, Z.; Jiang, L.; Su, M.; Feng, Z.; Zhang, L.; Wang, S.; Li, Z. and Hu, S. (2019). A new insight into lead (II) tolerance of environmental fungi based on a study of *Aspergillus niger* and *Penicillium oxalicum*. *Environmental Microbiology*. 21(1): 471-479.

Tian, D.; Wang, W.; Su, M.; Zheng, J.; Wu, Y.; Wang, S. et al. (2018). Remediation of lead contaminated water by geological fluorapatite and fungus *Penicillium oxalicum*. *Environmental Science and Pollution Research*. 25: 21118-21126.

Trivedy, R.K. and Goel, P.K. (1984). Chemical and Biological Method for Water Pollution. *Studies Environmental Pub, India*. 211-215.

Verma, R.K.; Chaurasia, L. and Katiyar, S. (2008). Potential antifungal plants for controlling building fungi. *Natural Product Radiance*. 7: 374-387.

Wainright, P.O.; Hinkle, G.; Sogin, M.L. and Stickel, S.K. (1993). Monophyletic origins of the Metazoa: an evolutionary link with fungi. *Science*. 260: 340-342.

Xu, X.; Hao, R.; Xu, H. and Lu, A. (2020). Removal mechanism of Pb (II) by *Penicillium polonicum*: immobilization, adsorption, and bioaccumulation. *Scientific Reports*. 10: 9079.

Xu, X.; Xia, L.; Huang, Q.; Gu, J. and Chen, W. (2012). Biosorption of cadmium by a metal-resistant filamentous fungus isolated from chicken manure compost. *Environmental Technology*. 33(14): 1661-1670.

Xu, C.; Ma, F. and Zhang, X. (2009). Lignin degradation and enzyme production by *Irpex lacteus* CD2 during solid state fermentation of corn stover. *Journal of Bioscience and Bioengineering*. 108(5): 372-375.

Yonnia, F.; Moreirab, M.T.; Fasolia, H.; Grandia, L. and Cabralc, D. (2004). Simple and easy method for the determination of fungal growth and decolourative capacity in solid media. *International Biodeterioration and Biodegradation*. 54(4): 283-287.

Zeng, G.M.; Wan, J.; Huang, D.L.; Hu, L.; Huang, C.; Cheng, M. et al. (2017). Precipitation, adsorption and rhizosphere effect: the mechanisms for phosphate induced Pb immobilization in soils-a review. *Journal of Hazardous Materials*. 339: 354-367.

Ziombra, M. (1994). Possibility of using ionizing radiation for growing *Pleurotus* sp. ESNA-XXIV annual meeting. *Book of abstracts*. Stara Zagora (Bulgaria). NaukaiTekhnika OOD. 124: 94-101.