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Bioremoval of Lead from Polluted Waters Using the Fungus *Talaromyces stipitatus* and Its Impact on Male Albino Rats

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ABSTRACT

Lead (Pb) is one of the most serious environmental pollutants due to its accumulation in soil and water, which causes serious problems for human health and the environment. Biosorption using dead microbial biomass represents one of the most innovative, economically feasible, and environmentally safe tools for removing heavy metals. Therefore, the present study focused on removing lead using *Talaromyces stipitatus* as a dead fungal biomass and evaluating its impact on animal toxicity. Lead-tolerant fungi were isolated from polluted sites, and *T. stipitatus* was chosen for the biosorption investigation based on its distinctive characteristics and identified according to its morphological and molecular characteristics then submitted to the GenBank database under accession number OQ691598. The biosorption capacity and efficiency of Pb were evaluated at 28±2°C with various parameters, which included pH, contact time and biomass concentration. Our findings showed that the highest significant biosorption capacity (56.10 mg/g) and efficiency (96.62%) for lead removal from aqueous solutions (lead initial concentration, 58 mg/L) were achieved at pH 6 and 30 minutes of contact time, with a biomass content of 1 g/L. Three groups of male albino rats were used after the biosorption study; the negative control group orally received double-distilled water only; the positive control group orally received Pb aqueous solution (58 mg/L) for 30 days, and the treatment group orally received Pb aqueous solution (58 mg/L) after being treated with *T. stipitatus* as biosorbent material for 30 days. The findings revealed that Pb induced a significant decrease in weight gain and relative weights of liver and testis, sperm count and motility, as well as serum total protein and albumin levels, but it induced a significant increase in relative weights of lung, brain, and prostate, serum aspartate aminotransferase and alanine aminotransferase activities, serum urea, creatinine, and uric acid levels, sperm abnormalities, and bioaccumulation of Pb traces in liver and kidney tissues with several histopathological changes in the liver, kidney, testis and epididymis of male albino rats. The treatment group showed considerable improvements in each of the aforementioned results in animals. These findings suggest that *T. stipitatus* is capable of removing lead from polluted waters without posing any health risks to animals. To the best of our knowledge, no previous report has documented the use of *T. stipitatus* for lead removal and its effect on animal toxicity.

INTRODUCTION

Due to their inertness and inaccessibility, heavy metal contaminations pose a serious risk to ecosystems across the globe **(Taha *et al*., 2023)**. **Volesky and Holan (1995)** recorded that, some heavy metals are a few examples of metal ions that are very harmful to animals and people with profound effects on the environment. Lead (Pb) is widely utilized in many industries, including the production of paints, ceramics and petroleum products despite being an environmental hazard. Lead is completely useless to the human body, and nowadays its pollution has become a big issue due to its harmful effects on various organs, which can result from its presence in the body at any age, in either sex, or through any route of exposure **(Rashed *et al*., 2021)**.

When heavy metal concentrations are low, conventional techniques of removal (such as precipitation, filtration, ion exchange, carbon adsorption, evaporation membrane technology, etc.) have shown to be unsuccessful and nonspecific **(Ali *et al*., 2019; Taha *et al*., 2023)**. To counter these drawbacks of conventional heavy metal removal techniques, biosorption (biological green technology) has emerged as a promising new approach **(Li *et al*., 2018)**. Among the advantages of biosorption, its simplicity, low cost, increased efficiency, high sensitivity, minimal technology requirements, and low amount of chemical sludge production are regarded **(Gouda & Taha, 2023)**.

The use of microorganisms (bacteria, fungi and microalgae) as adsorbent materials has gained attention in recent years due to advancements in microbial biotechnology. Fungi have gained a lot of interest as a viable alternative approach for the removal of water contaminants because of the carboxyl, phosphate and amine hydroxyl groups present on the surface of their cells **(Legorreta-Castañeda *et al*., 2020)**, and they are produced in large quantities as wastes of various industrial processes. Several fungal species, such as *Alternaria alternata* and *Trichoderma harzianum* **(Zghair & Jebar, 2020)**, *Aspergillus piperis* **(De Wet & Brink, 2021)**, *Aspergillus tubingensis* **(Shan *et al*., 2022)**, and *Rhodotorula mucilaginosa* **(Tian *et al*., 2022)**, have all been employed to remove lead in various investigations.

The genus *Talaromyces* is an ascomyceteous fungus (**Kirk *et al*., 2001**). It has a worldwide distribution in soil, detritus, manure, agricultural or industrial wastes, dung, and other substrates. Due to its resistance to extreme conditions, several *Talaromyces* species have been exploited in industrial and environmental biotechnology research. They also offer prospective biomasses for use in absorptive technology **(Okparanma & Ayotamuno, 2008; Katar *et al*., 2017)**. *T. helicus* has been frequently utilized to eliminate cobalt, copper and cadmium from polluted sediments of industries **(Guerra Sierra *et al*., 2022)**. While, *Talaromyces amestolkiae* has been used to treat uranium-affected water **(Bengtsson *et al*., 1995; Coelho *et al*., 2022)**.

There are no more reports of using *Talaromyces* dead biomass in lead removal. Therefore, the objectives of this research were (i) the isolation of a biosorbent from polluted sites that was used for removal of Pb from aqueous solutions; (ii) the determination of lead biosorption capacity and efficiency from aqueous solutions under different conditions (e.g. pH, contact time and concentration of biomass); (iii) the assessment of the effects of lead biosorption on general health, semen quality, relative organ weights, some biochemical parameters, bioaccumulation of Pb on liver and kidney tissues and histological alterations (liver, kidney, testis and epididymis) in male mature Wistar albino rats.

MATERIALS AND METHODS

1. Lead solutions preparation

Stock lead solution (1000 ppm) was made in double distilled water using extra pure lead nitrate [$Pb(NO_3)_2$] purchased from Alpha Chemika™ (Mumbai, India). The stock solution was kept in a sealed flask at 4 degrees Celsius. The preparation of working solutions was done by diluting the stock solution with sterile, double-distilled water and filter-sterilized with bacteriological filters.

2. Fungal isolation

Pb-tolerant fungi were isolated using a standard serial dilution method on potato dextrose agar (PDA) media, containing dextrose (20 g), agar (20 g), potato slices (200 g) and double distilled water (1000 mL). Water and soil were sampled from polluted sites (at Ismailia canals and soil sited surrounding it) in clean, sterilized containers in ice boxes. Water (1 mL) and soil (1 g) samples were diluted to 10 mL with sterile double distilled water. Aliquot (100 µL) of diluted water and soil was equally spread on PDA plates supplemented with filter sterilized $Pb(NO_3)_2$ at 25 ppm. Then, plates were incubated at 28±2°C **(Joshi *et al*., 2011)**. The fungal isolate used in this study was selected due to its distinct morphology that developed after 7 days on PDA media and was purified on the same media using the zigzag technique. The pure cultures of this fungus were kept on PDA slants at 4 degrees Celsius to be used for further investigations and given the code name Tal1 until identification (**Bahobil *et al*., 2017)**.

3. Fungal identification

Firstly, the isolated fungus Tal1 was identified at the genus level according to its micro- and macro-morphology by observing colony growth, color and sporulation for a 7-day incubation at 28±2°C on PDA media and using the slide culture procedure for observing the septation of mycelia and spore shapes as well as their color under a light microscope at 100× and 400× magnification. Morphological identification was additionally confirmed by molecular identification. Genomic DNA of isolate Tal1 was extracted using Quick-DNA™ Fungal Microprep Kit (Zymo Research) according to manufacturers' protocol as described by **Geweely *et al*. (2023)**. The fungal D1/D2 region

of large ribosomal subunit (28S rRNA) gene was amplified by PCR using NL-1 (forward) 5′-GCA TAT CAA TAA GCG GAG GAA AAG-3′ and NL4 (reverse) 5′-GGT CCG TGT TTC AAG ACG G-3′ primers **(Kwiatkowski *et al*., 2012)**. The PCR program was performed at an initial denaturation step at 95°C for 2 min, followed by 35 cycles at 95°C for 15 s, 50°C for 20 s and 72°C for 1 min, and then a final extension step at 72°C for 5 min. The PCR product was sequenced at GATC Company, German, by using ABI 3730x1 DNA sequencer. The obtained sequence of isolate Tal1 was used to carry out a basic local alignment search tool (BLAST; http://www.ncbi.nlm.nih.gov website) with database of GenBank of the National Center for Biotechnology Information (NCBI) **(Altschul *et al*., 1997)**. Nucleotide sequencing data for the isolated fungal strain Tal1 was submitted to the NCBI GenBank database. The neighboring join (NJ) method in molecular evolutionary genetics analysis (MEGA 11) was used to build the phylogenetic tree, and bootstrap of 1000 replicates was calculated **(Felsenstein, 1985; Tamura *et al*., 2021)**.

4. Lead tolerance index test

Further screening was performed to determine the tolerance index (TI) of *Talaromyces stipitatus* (Tal1). The test was conducted by inoculating *T. stipitatus* agar plug (7 mm) into the center of plates containing PDA medium with various concentration of $Pb(NO_3)_2$ at 25, 50, 100, 200, and 400 ppm, compared to control plates (without heavy metal). Then, plates were incubated at 28±2°C for 7 days, and mycelial radial growth was recorded. Tolerance index (TI) of *T. stipitatus* was calculated in relation to the radial growths of control by equation (1) **(Valix & Loon, 2003; Anahid *et al*., 2011)**. Fungal heavy metal tolerance was categorized based on TIs as follows; 0 indicates sensitive; 0 > TI > 0.29 indicates very low resistance; 0.30 > TI > 0.49 indicates low resistance; 0.50 > TI > 0.69 indicates medium resistance; 0.70 > TI > 0.89 indicates high resistance, and TI > 0.90 indicates extremely maximum resistance (**Văcar *et al*., 2021)**.

$$\textbf{Tolerance index (TI)} = \frac{Dt}{Du} \quad \ldots\ldots (1)$$

Where,

Dt = Radial growth of *T. stipitatus* grown in Pb amended medium (mm)

Du = Radial growth of *T. stipitatus* grown in control (mm)

5. Production and preparation of biosorbent

Talaromyces stipitatus agar plugs were inoculated into potato dextrose broth (PDB) medium in Erlenmeyer flasks and then fermented at 28±2 degrees Celsius in a shaking incubator at 125 rpm to produce biomass. After incubation for 7 days, the fungal growth was filtered through Whatman No.1 filter paper. The biomass was collected, then autoclaved for 15 minutes at 121ºC after being rinsed many times in sufficient sterile double-distilled water. Afterward, it was dehydrated at 50 degrees Celsius until dryness.

A mortar and pestle were used to smash and grind the dry, dead biomass into powder **(Słaba & Długoński, 2011; Mali *et al*., 2014)** to use for biosorption assays.

6. Biosorption study and analytical methods

The biosorption was carried out following the study of **Soleimani *et al*. (2016)**, with modifications. The effect of initial pH, contact time and biomass concentration on the biosorption of Pb was investigated by adding 100 mL of a lead aqueous solution at a fixed concentration to 250 mL flasks and agitating the mixture on a rotatory shaker (125 rpm) at room temperature (28±2°C) in a series of batch adsorption experiments. The effect of pH on Pb adsorption was investigated by conducting experiments, with metal solutions of pH 4, 5 and 6. To adjust the pH of each solution, either 0.1 N NaOH or 0.1 N HCl was added. At optimum pH, samples were collected after 15, 30 and 60 minutes **(Nofiani *et al*., 2022)**. Three different biomass concentrations (1, 2 and 3 g/L) were tested at the optimal pH and contact time. The biomass concentrations were determined according to the data published by **Rao and Bhargavi (2013)** and **Aracagök *et al*. (2021)**. Flame atomic absorption spectrophotometry (AAS; Savant AA; GBC scientific equipment) was used to determine the amounts of lead ions in lead solutions before and at the end of each experiment, as recommended by the method of the American Public Health Association **(APHA-3111B, 2017)**. Each test was repeated thrice, and the average was chosen as the best result via statistical analysis. Both the biosorption capacity (q) in mg/g of dead biomass and the biosorption efficiency (%) were determined using equations 2 and 3, respectively **(Fan *et al*., 2008; Javaid *et al*., 2010)**.

$$q\ (mg/g) = V\ (C_i - C_f)\ /m \quad \ldots\ldots (2)$$

$$\text{Biosorption efficiency } (\%) = (C_i - C_f)/C_i \times 100 \quad \ldots\ldots (3)$$

Where, q is the biosorption capacity (mg metal/g dried dead biomass); C_i and C_f are the lead ion initial and final concentration in supernatant solutions (mg metal/L), respectively; m is the mass of the biosorbent (g), and V is the volume of metal solution (L).

7. Experimental animals

The National Research Center in Cairo, Egypt provided fifteen male, adult Wistar albino rats (*Rattus norvegicus*) weighing 118.24±1.86 g. At the Animal Care Unit of the Zoology Department, Faculty of Science, Ain Shams University in Cairo, Egypt, the rats were kept in suitable cages. Ten days before the commencement of the study, they were provided with a healthy diet of normal rodent feed, water and comfortable housing for acclimatization to laboratory settings including exposure to natural light and dark cycles; temperatures between 22.2 and 28.5 degrees Celsius, and relative humidity between 40 and 60 percent. This work was approved by the Research Ethics Committee of the Faculty of Science at Ain Shams University in Cairo, Egypt **(Approval Code: ASU-SCI/ZOOL/2023/8/2)**. Every effort was made to alleviate the rat's pain. This limited the number of animals that could be utilized to provide accurate research results.

8. Experimental design and procedure

Following ten days of acclimatization, rats were randomly allocated to three groups, with five rats in each group as follows.

- Normal control group (GI): Rats were orally given 1.0mL of double-distilled water as a vehicle for 30 days.
- Pb-toxic group (GII): Rats were orally given 1.0mL of a prepared aqueous solution of Pb-nitrate (58 mg Pb/L) for 30 days.

-Treatment group (GIII): Rats were orally given 1.0mL of a prepared aqueous solution of Pb-nitrate (58 mg Pb/L) after being treated with dead fungal biomass (*Talaromyces stipitatus*) as a biosorbent material for 30 days.

9. Clinical observations and body weight evaluation

Throughout the experimental period, every rat was checked twice daily for symptoms of mortality and once daily for clinical toxicity. The weight of each rat group was measured using an automated balance before starting (initial body weight) and after (body weight on the day of sacrifice) (final body weight) treatments. Determination of changes in body weight as a percentage was calculated according to formula (4):

Body weight change %= {(final body weight-initial body weight/initial body weight)×100}……....(4)

10. Blood and tissue sampling

Blood samples were taken through heart puncture and centrifuged after being allowed to clot (at 1,800 ×*g* for 10 minutes). Biochemical parameters were measured after storing serum supernatants (at –20°C) for determining liver and kidney functions. Anesthetization of rats were done by using chloroform. The necropsy resulted in the removal, dissection and partitioning of the kidney and liver into two parts. One part was kept in neutral-buffered formalin at 10 percent for histological examination, while the other was kept at –20°C for analysis of Pb trace element residues. The right sides of liver, kidney, testes and epididymis were dissected and examined histologically. The semen quality was determined by placing the left epididymis in 2mL of buffered saline (NaCl 0.9 percent) at 35ºC. The tissues of scarified rats were examined for obvious gross alterations. Before being weighed, internal organs such as liver, kidney, heart, lung, and spleen as well as the testis, epididymis, vesicula seminalis and prostate gland were cleaned of any sticking tissues and blood. The following formula was used to determine the relative weights of the different organs:

(Absolute organ weight/sacrificed body weight)X100

11. Biochemical assays

Methods of **Bergmeyer *et al*. (1978)** were implemented to assess aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities. Total protein (TP) level in serum was determined using the method of **Yatzidis (1987)**. In addition, serum albumin (Alb) concentrations were determined based on the standard of **Doumas *et al* (1971)**. The reagent kits used to analyze ALT and AST activities and TP and Alb levels

were purchased from the Egyptian Biotechnology Company Spectrum in El Obour City (Cairo, Egypt). Kidney functions were determined by measuring serum concentrations of uric acid, creatinine and urea. Creatinine and urea concentrations were measured by colorimetric methods of **Henry *et al*. (1974)** and **Patton and Crouch (1977)**, respectively. **Whitehead *et al*. (1991)** detailed a method for determining the serum uric acid concentrations. Laboratory reagent kits for measuring serum urea, creatinine and uric acid were acquired from Bio Diagnostic in Dokki (Giza, Egypt).

12. Detection of lead residue in liver and kidney tissues

Following the protocol specified by the American Public Health Association, the livers and kidneys of rats were removed, frozen in an ice box for lead analysis performed by using a Flame Atomic Absorption Spectrophotometer (Savant AA, GBC Scientific Equipment) **(APHA-3111B, 2017)**. For the study of lead levels, five samples were examined, and average values were recorded. Before analysis, liver and kidney samples were digested in vessels with one gram of the ground samples and 10mL of concentrated nitric acid (HNO_3) using the CEM Microwave Digestion system (MDS-2000, USA). It took at least one night for the reaction to take place in these vessels. After that, a turntable hooked up to the system was used to rotate the containers while the heating program was let to run until digestion was complete. After the samples were cooled for 5 minutes, the turntable was removed from the system.

For the lead analysis, 25mL of distilled water was added to the digested samples and put in a Flame Atomic Absorption Spectrophotometer. At a certain wavelength and slit width, lead concentration was determined. The resulting tissue concentration was reported as mg/kg using the following formula:

$$\text{Concentration (mg/kg)} = \frac{\text{Concentration (mg/L) X Volume (mL)}}{\text{Weight (g)}}$$

13. Semen quality assessment

To facilitate sperm release, left epididymis was minced and put on a petri plate containing 2ml of buffered saline (NaCl 0.9 percent). A hemocytometer of the Neubauer type was used to examine semen under the microscope. Cells on the top and right limits of the squares were disregarded while counting sperm in 4 corner squares and the center square. Each rat had their sperm count done at least five times, and the results were averaged to reduce error. The number of sperm per milliliter was used to represent the data. A light microscope with 400x magnification was used to determine the sperm's percentage of motility. The levels of sperm motility were classified into three groups. There are three types of sperm: 1) progressive motile sperm means that are motile and move in either straight lines or huge circles; 2) non-progressive motile sperm; means that are motile and either move in place or very tiny circles; and 3) immotile sperm; means that are immotile and don't move at all. The motility of each sample was measured in three separate fields, and a mean value was then reported. The same samples evaluated for sperm motility were also used to measure sperm morphology. Sperms were smeared over microscope slides, air-dried, and then preserved with methanol. Aqueous eosin Y (1 percent w/v) was used to stain the samples for 1 hour after they were fixed, washed with distilled water, dehydrated, cleared and mounted in neutral resin beneath a coverslip. One

hundred spermatozoa were examined at a magnification of 1,000× using a light microscope with an oil immersion objective lens for each sample **(Mahmoud *et al*., 2018; Taha & Soliman, 2019)**. The morphology of sperm was evaluated in terms of its proportion to the total number of spermatozoa. The sperm were deemed abnormal by **WHO (2000)**.

14. Histological examination

The right side of samples of each rat's liver, kidney, testis and epididymis were immersed in 10% neutral-buffered formalin, washed, dried, clarified and embedded in paraffin. Hematoxylin and eosin staining was performed on 5µm-thick sections (H&E). Synthetic resin mounting medium comprised of distyrene, a plasticizer and xylene was used to dip the slides in before they were covered with a coverslip (DPX) **(Taha, 2022; Taha & Gouda, 2022)**. A light microscope was used to examine the slides.

15. Morphometric measurements of testis and epididymis and Johnsen's score of spermatogenesis

Magnification of 400× was used to measure the diameter and height of seminiferous tubules in the testis and the diameter of epididymal ducts in the epididymis in ten non-overlapping randomly chosen fields. The spermatogenesis phases are quantified using Johnsen's scoring system. Each tubule is scored on a scale from ten to one based on the criteria of **Celik *et al.* (2013)**. Number ten represents the completion of spermatogenesis; nine stands for minimal spermatogenesis impairment; while, number 8 indicates that there are less than five spermatozoa in each tubule. Seven indicates a large number of early spermatids but no late ones; six represents the stopping of spermatogenesis process at phase of the spermatid; five indicates the presence of lot of spermatocytes; four represents minimal of spermatocytes; three degree represents the stoppage of spermatogenesis in its early stages; two degree represent presence only of spermatogonia, and one indicates no germ cells, Sertoli cells only, and almost empty lumen. A rating is inferred by randomly selecting 10 seminiferous tubules for each rat.

16. Statistical analysis

All data values were tabulated and statistically evaluated using one-way analysis of variance (ANOVA), followed by Tukey's *post hoc* multiple comparisons test for comparative analysis, using Minitab V17 for Windows (Minitab Inc., USA). The results were displayed as mean± standard error (SE) and statistically significant difference at a *P*-value of less than 5%.

RESULTS AND DISCUSSION

The biosorption of heavy metals by biological materials is a potential method for cleaning the environment. Microbial biomass as biosorbents for the removal of heavy metals is a relatively new concept in the environmental biotechnology field. Fungi were chosen for this study because of the unique properties of the fungal cell wall surface that gather metals and their ability to be produced on a large scale using low-cost growing

media, making them practical in the bioremoval of heavy metals from liquid substrates **(Konopka *et al*., 1999; Gouda & Taha, 2023)**.

1. Morphological and molecular identification of isolated fungus

Numerous fungal species have been found in contaminated environments with high levels of heavy metals. It's well known that exposing water and soil to heavy metals over extended periods of time may drastically alter the microbial communities present there **(Jansen *et al.*, 1994)**. In this study, the fungus Tal1 was isolated from polluted sites and selected due to its distinct features, then morphologically identified as a member belonging to genus *Talaromyces.* The colonies of isolated fungus *Talaromyces* Tal1 were plane, bright yellow and slightly rose at the center. The conidiophores were biverticillate and monoverticillate, carrying ellipsoidal to ovoidal shaped conidia. Ascomata was developed and characterized by its ellipsoidal ascospores that are smooth, flattened, and have a single equatorial ridge (Fig. 1).

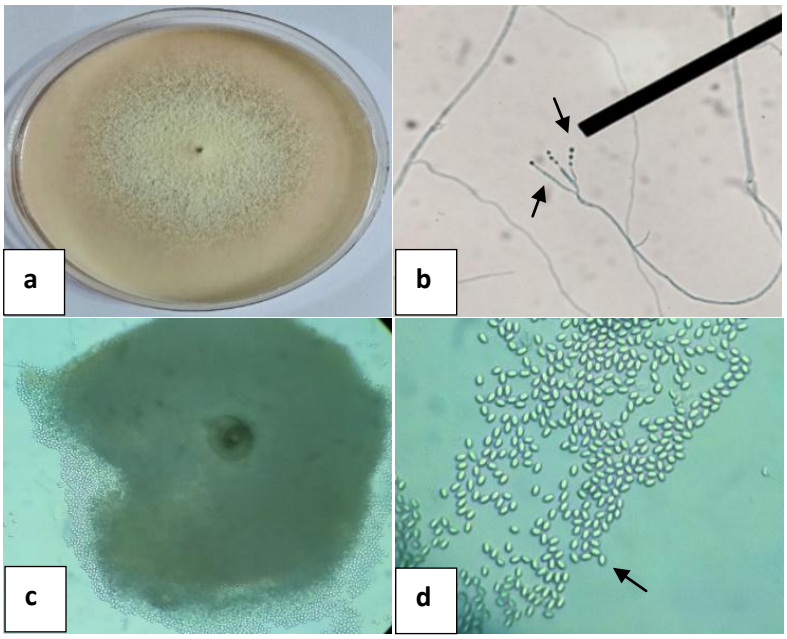

Fig. 1. Morphological characteristics of *Talaromyces* Tal1 showing: (a) Colony growth on potato dextrose agar (PDA) media at 28±2°C for 7 days, (b) Conidiophore and conidia (arrows), (c) Ascomata, and (d) Ascospores (arrow) at 400×.

In the current investigation, morphological identification was confirmed by molecular identification. Based on the sequence obtained from molecular identification together with its morphological features, the fungal isolate Tal1 was identified as *Talaromyces stipitatus* and recorded in GenBank under the accession number of OQ691598. The phylogenetic tree of *Talaromyces stipitatus* Tal1 with closely related species sequences data from NCBI was constructed using the neighboring join (NJ) method to analyze their evolutionary relationships, as shown in Fig. (2). *Talaromyces stipitatus* was genetically determined by sequencing the ribosomal DNA from the LSU-D1/D2 region. This region has been used for fungal identification before, as described by **Liu *et al*. (2012)**. Since the internal transcribed spacer (ITS) and D1/D2 domains of rDNA

are more reliable and objective than other genetic regions, they have been used for fungal identification. To date, no other genetic region has been found to be more capable of differentiating between species than the D1/D2 domain **(Abliz *et al*., 2004)**.

Talaromyces sp. has been demontorated in earlier studies to adsorb metal ions. Nam and others have employed *Talaromyces* sp. KM-31, isolated from polluted mine soil for arsenic (As) adsorption investigations **(Nam *et al*., 2019)**. *T. helicus* can detoxify and adapt to heavy metals, and it has been used to break down biphenyl treated with high concentrations of copper (Cu) **(Romero *et al*. 2006)**. Uranium (U) biosorption has also been reported using *T. emersonii* **(Bengtsson *et al*., 1995)**. Therefore, we used *T. stipitatus* for lead biosorption in this investigation.

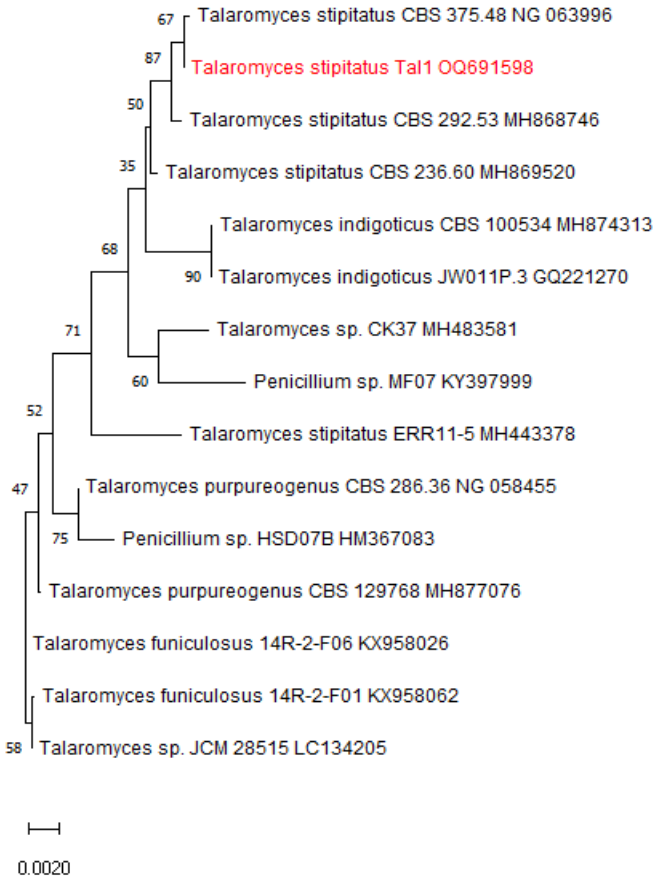

Fig. 2. Neighbor-joining tree based on the D1/D2 region sequences showing the phylogenetic relationship of *Talaromyces stipitatus* Tal1 (OQ691598) in red color and other related gene sequences. Bootstrap values were computed over 1000 replicates.

2. Lead tolerance index test

To evaluate the impact of metals on fungal development, the tolerance index (TI) was extensively used **(Rose & Devi, 2018)**. On exposure to all lead concentrations, *Talaromyces stipitatus* exhibited inhibited mycelial radial growth that differed significantly compared to the control (Fig. 3), followed by tolerance index calculations of

the test fungal isolate in relation to its controls using an average of the mycelial growth of three replicates on lead-containing media. *T. stipitatus* exhibited a high tolerance to lead at 25, 50 and 100 ppm with TI values of 0.88, 0.84 and 0.79, respectively, and a moderate tolerance to lead at 200 and 400 ppm (TI; 0.68 and 0.58, respectively). The growth of *T. stipitatus* decreases with increasing lead concentrations. This is related to the inhibitory effect of heavy metals on growth, as seen by the lower TI **(Ge *et al*., 2011)**. Many fungal species were shown to be metal-tolerant **(Qazilbash, 2004; Turnau *et al*., 2006)** and even flourish in high-metal environments **(Adriaensen *et al*., 2005; Anand *et al*., 2006)**. The prevalence of filamentous fungus in polluted areas, as revealed by *Talaromyces* identified here, suggests that these organisms have adapted to harsh soil conditions and developed particular mechanisms for such resistance. **Vala and Sutariya (2012)** found that the mechanisms exerted by fungi to tolerate metals at high concentrations include cell wall binding, intracellular and extracellular enzymes production, sequestration within the cell, extracellular sequestration and precipitation, metal influx inhibition, increased metal efflux and complexation. **Vepachedu *et al*. (1997)** and **Malik (2004)** reported that higher amounts of heavy metals have deleterious effects on bacterial and fungal growth. To avoid this effect, the dead fungal biomass of *T. stipitatus* was used in this study as a biosorbent for lead biosorption.

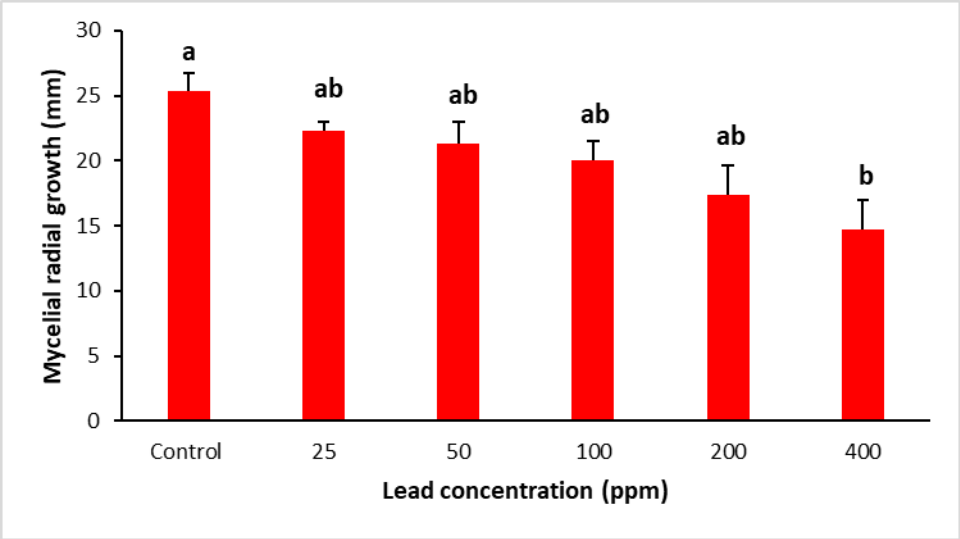

Fig. 3. Effect of varied concentrations of lead (Pb) on *Talaromyces stipitatus* radial growth (mm) after 7 days of incubation. Data are expressed as means of 3 replicates ± SE. Bars with different letters are significantly different ($P < 0.05$) according to Tukey's multiple comparison test.

3. Biosorption study

The cell wall structure of fungi contains functional groups such as carboxyl, amine hydroxyl, phosphate, and sulfhydryl, making fungi a potential biosorbent for the removal of heavy metals through a range of absorptive mechanisms **(Legorreta-Castañeda *et al*., 2022; Gouda & Taha, 2023)**. Physical treatment (including boiling, autoclaving and freeze-drying) or chemical treatment that modifies the biosorbent's metal affinity, permeability and surface charges may improve metal biosorption using fungal biomass **(Wang & Chen, 2009)**. In our research, *T. stipitatus* had a greater accessible surface area

with more surface binding sites due to the disintegration of the cell wall after heat inactivation by autoclaving **(López & Vázquez, 2003)**. For the purpose of increasing the biosorbent's biosorption ability, the dried, dead biomass was powdered to get the lowest particle size, giving a higher surface area and avoiding particle aggregation simultaneously **(Bahobil *et al*., 2017)**. Metal biosorption processes by fungi are significantly influenced by environmental parameters, such as pH, contact duration and biomass content **(Ghosh *et al*., 2016; Gouda & Taha, 2023)**.

3.1. Effect of initial pH

The biosorption process is strongly influenced by the initial pH value of the heavy metal solution **(Ali *et al*., 2021; Gouda & Taha, 2023)**. With respect to this parameter, the highest significant biosorption capacity (15.933± 0.0987 mg/g) was observed at pH value of 6 with a biosorption efficiency of 82.327±0.766%. Therefore, all other experiments were done at a pH 6. In contrast, low pH (4) had a more significant reducing effect on the lead biosorption capacity (9.8167±0.0884 mg/g) and efficiency (50.74±0.212%), as shown in Fig. 4. Binding sites on biomass, such as hydroxyl, carboxyl, sulfhydryl, sulfonate and phosphonate are responsible for these findings. In aqueous solutions at low pH, H^+ ions compete with metal ions (Pb) and reduce Pb biosorption when the binding sites are protonated or present in neutral form. However, when the initial pH rises from 4 to 6, the proportion of deprotonated and binding sites with negative charge on biomass rises, resulting in a greater Pb biosorption capability **(Silva *et al*., 2017; Mohapatra *et al*., 2019; Nofiani *et al*., 2022)**. These findings are consistent with those of **Wang and Chen (2006)**, **Farah *et al. (*2007)** and **Gouda and Taha (2023)** who found that, the biosorption effectiveness is high at moderate pH and low at higher pH owing to the formation of metal complex precipitants, which might impede the sorption process and was thus avoided in our research.

In agreement with our findings, **El-Sayed and Reda (2011)** found that *Cunninghamella elagans* had a maximum lead biosorption (48.70 mg/g) at a pH value of 6. In addition, **Farhan and Khadom (2015)** showed that *Saccharomyces Cerevisiae* was effective in removing Pb from aqueous solutions at pH 6.0. For *Aspergillus versicolor*, *Rhizopus oligosporus* and *Penicillium purpurogenum*, the highest metal biosorption was observed at pH 6.0 and decreased above pH 6.0 **(Juárez *et al*., 2012)**. In contrast, other studies have found that the biosorption of ions uranium (VI), cadmium, copper, lead, iron and manganese utilizing *Fusarium* sp., *Rhizopus cohnii*, *Rhizopus arrhizus*, *Penicillium chrysogenum*, *Pleurotus mutilus*, and *Aspergillus niger* was most effective at low pH (2.0 to 4.5) **(Júnior *et al*., 2003; Yang *et al*., 2011; Adeogun *et al*., 2012; Madani *et al*., 2015)**.

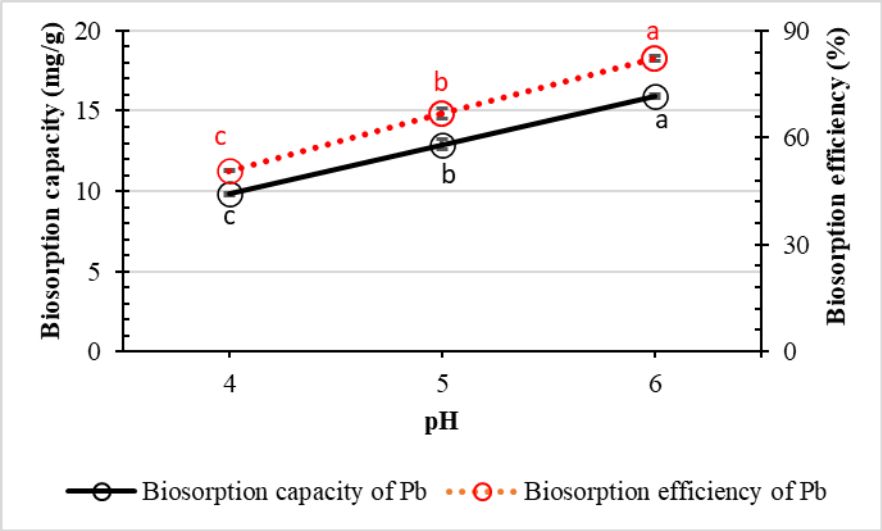

Fig. 4. Effect of initial pH on the biosorption capacity and efficiency of Pb (initial concentration 58 mg/L, contact time 30 minutes, and biomass concentration 3 g/L) using *T. stipitatus* dead biomass. Data are expressed as means of 3 replicates ± SE. Bars with different letters are significantly different ($P < 0.05$) according to Tukey's multiple comparison test.

3.2. Effect of contact time

The contact time is crucial when determining whether this fungal biomass is suitable biosorbents in a continuous flow system or not. Biosorption tests were run at 15, 30 and 60 minutes to determine the impact of contact (sorption) time at a pH value of 6. Our results in Fig. 5 show that the biosorption capacity of Pb has likely reached its highest significant value of 16.027±0.0888 mg/g with a biosorption efficiency of 82.82±0.279 at a sorption time of 30 min. It was observed that after 30 min, the biosorption efficiency decreased significantly to 34.84±1.08% at a sorption time of 60 min. According to **Kumar *et al.* (2020)**, the biosorption pattern over time results in the sites on the biomass surface being saturated and unusable for more adsorption through adsorption, complexation, binding, or ion exchange. In line with our results, the Pb biosorption capacity of *Cunninghemella elegans* increased rapidly with time and reached its maximum at 30 min (**El-Sayed & Reda, 2011**). Moreover, **Khodabakhshi *et al.* (2022)** and **El-Gendy *et al.* (2023)** revealed that the highest Pb adsorption using nonliving fungal biomass occurred in the first 30 min. Other studies recorded that 95% of lead ions were adsorbed by different fungal species within the first 90 min **(Bueno *et al.*, 2008; Sánchez-Castellón *et al.*, 2022)**.

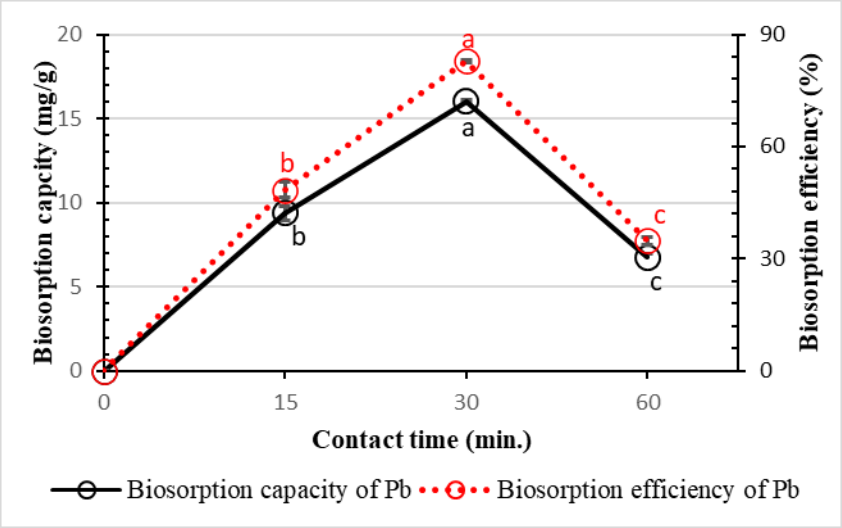

Fig. 5. Effect of contact time on the biosorption capacity and efficiency of Pb (at pH 6, initial concentration 58 mg/L, and biomass concentration 3 g/L) using *Talaromyces stipitatus* dead biomass. Data are expressed as means of 3 replicates ± SE. Bars with different letters are significantly different ($P < 0.05$) according to Tukey's multiple comparison test.

3.3. Effect of biomass concentration

Better biosorption of lead was achieved by further optimizing the fungal biomass concentration. Fig. 6 reveals that the maximum significant lead biosorption capacity of 56.10±0.372 mg/g was achieved at 1 g/L of *T. stipitatus* biomass, with a biosorption efficiency of 96.62±0.189 percent, and that both values declined with increasing biomass concentration to 3 g/L. Both **Iskandar *et al.* (2011)** and **Gouda and Taha (2023)** reported that the concentration of the biosorbent plays a crucial role in the biosorption process. This is attributed to the number of binding sites on the biosorbent's surface and the electrostatic forces between the cells of the biosorbent **(Mali *et al.*, 2014; Farhan & Khadom, 2015)**. A higher metal to biosorbent ratio may account for the greater adsorption seen at lower biomass concentrations. Since there are more metal binding sites on the fungal biomass surface than metal ions, and since the shell effect prevents the binding sites from being filled by metal ions, the sorption decreases as the concentration of biomass rises **(Aryal *et al.*, 2012)**.

Previous studies supported our findings considering that the biosorption capacity of cadmium, zinc, and lead decreases as *Aspergillus niger*, *Penicillium simplicissimum* and *Fennelia nivea* biomass increases **(Júnior *et al.*, 2003; Fan *et al.*, 2008; Aracagök *et al.*, 2021)**. However, **José *et al.* (2019)** found that an increase in biomass improves the capacity to remove metals, which is related to the number of metal-accessible binding sites on the amount of additional biosorbent. According to another research, when the amount of *Pleurotus ostreatus* biomass increases, the amount of metal ions removed also increases **(Javaid *et al.*, 2011)**.

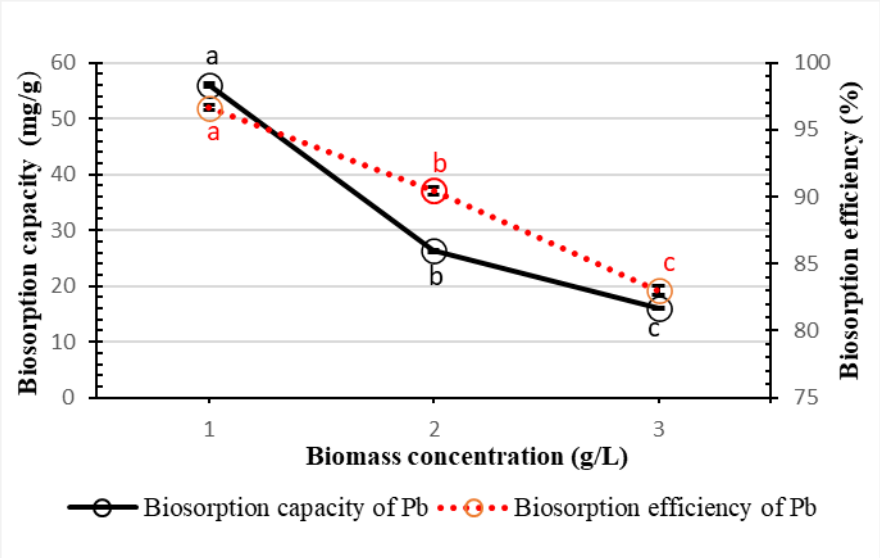

Fig. 6. Effect of biomass concentration of *T. stipitatus* on the biosorption capacity and efficiency of Pb (pH 6, initial lead concentration 58 mg/L, and contact time 30 minutes). Data are expressed as means of 3 replicates ± SE. Bars with different letters are significantly different ($P < 0.05$) according to Tukey's multiple comparison test.

4. Effect of lead removal using *T. stipitatus* on clinical observations and body weight gain in albino rats

During the experimental study, rats in all treatment groups did not exhibit serious signs of toxicity. There was no mortality recorded during the experimental period. In the present study, rats of the Pb-toxic group (GII) showed general body weakness and reduced appetite which corresponded to significant body weight loss ($P<0.001$), as compared with untreated ones (GI) **(**Table 1). This is attributed to the harmful effect of Pb on body weight gain **(Ibrahim *et al*., 2012)**. The harmful Pb-ions may be to blame for this weight loss, which has been linked to a number of variables that upset the body's metabolism and reduced zinc levels in enzymes critical to several cellular functions **(Ibrahim *et al*., 2012)** that impair intestinal absorption of some essential trace elements **(Ramah *et al*., 2015)**. After receiving fungal treatment (GIII), rats behaved similarly to untreated rats, consuming the same quantity of food and water. Gains in body weight in this group (GIII) were significantly higher ($P <0.001$) than in the Pb-toxic group (GII), and eventually reached the normal range (Table 1). The results suggested that lead biosorption by fungi was to blame for the observed rise in weight gain.

Table 1. Effect of lead removal using *T. stipitatus* on body weight gain in male albino rats.

Group	Initial body weight (g)	Final body weight (g)	Percentage of change in body weight	The gain to normal control
GI	113 ± 2	174 ± 6.78	54.07 ± 6.1	100
GII	120.6 ± 1.16	131.6 ± 3.9	9.19 ± 3.6†	17
GIII	114.6 ± 1.28	153.8 ± 2.22	34.3 ± 3.1*	63.45

Data are expressed as mean ± SE. †Symbol represents significance compared with the negative normal control group, where: † $P< 0.05$. *Symbol represents significance compared with the Pb-toxic group, where: *: $P< 0.01$.

5. Effect of lead removal using *T. stipitatus* on biochemical analysis in male albino rats

Compared to the control group (GI), ALT and AST activities were significantly increased in the Pb-toxic group (GII) **(**Table 2**)**. These results might be due to the increased liver microsomal membrane fluidity, free radical production and liver changes caused by the heavy metal exposure **(Abdou *et al*., 2007)**. This might be because of the widespread breakdown of bodily tissue or because these enzymes are generated as liver damage and necrosis proceed **(El-Nekeety *et al*., 2009)**. Previous research linking lead's hepatotoxicity to an increase in ALT and AST serum activity is supported by our findings **(Ibrahim *et al*., 2012; Ibrahim *et al*., 2014; Hannah *et al*., 2016)**. The Pb-toxic group (GII) had significant lower total protein and albumin levels than the control group (GI). Changes in serum total protein concentrations may be a sign of liver disease since the liver is the principal place for plasma protein synthesis, particularly albumin **(Burtis & Bruns, 2015)**. Changes in the total protein concentration of plasma were related to fluctuations in albumin value. The decline in plasma total soluble protein and albumin levels may be due to the low significant excretion of hormones that regulate protein creation and certain enzymes in cellular activities **(Murray *et al*., 2006)**. Creatinine, urea and uric acid levels were all significantly higher in the Pb-toxic (GII) group compared to the control group (GI; $P< 0.001$) **(**Table 2**)**. These results suggest that lead's effect on kidney function is due to the metal's ability to alter the antioxidant defense system, which in turn causes kidney damage **(Ibrahim *et al*., 2012)**. According to earlier studies **(Abdel-Wahhab *et al*., 2008)**, Pb-intoxication is linked to a drop in serum total protein and an increase in blood urea, which may be indicative of protein catabolism and renal failure. When exposed to lead, plasma albumin and soluble proteins may precipitate. The majority of inorganic lead (9%) is present in plasma, where it inhibits plasma cholinesterase activity and alkaline phosphatase and is associated with alterations that contribute to

kidney toxicity and injury, as reported by **Antinio *et al*. (2003)**. Hypothesized lead treatments decreased copper and zinc in the liver, two essential cofactors for antioxidant enzymes **(Ibrahim *et al*., 2012)**. These results established beyond a reasonable doubt that lead is toxic to and stressful for the liver and kidneys.

Total protein and albumin were significantly higher in the treatment group (GIII) compared to the Pb-toxic group (GII), and serum AST and ALT activity, creatinine, urea and uric acid were significantly lower than the Pb-toxic group (GII) (Table 2). According to these findings, biochemical markers measuring liver and kidney function in rats were not negatively affected by fungal biosorption of lead.

Table 2. Effect of lead removal using *T. stipitatus* on liver and kidney functions for male albino rats.

Group	Hepatic biochemical parameters				Kidney function parameters		
	ALT activity (U/L)	AST activity (U/L)	Total protein (g/dL)	Albumin (g/dL)	Creatinine (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)
GI	48.6 ± 6.2	111.2±0.58	8.28±0.11	4.2±0.13	0.8 ± 0.031	28.8 ±1.39	2.84 ± 0.05
GII	74±1.9†	161.8±9.50††	7.02±0.18†††	3.4±0.22†	1.2 ± 0.167†	45.2 ± 5.5†	3.26 ± 0.12††
GIII	54.4±5.8*	127.8±0.96*	8.02±0.139*	4.28±0.007*	0.78 ± 0.03*	27.2 ±0.58*	2.92 ± 0.058*

ALT: alanine aminotransferase; AST: aspartate aminotransferase. Data are expressed as mean ± SE. †Symbol represents significance compared with the negative normal control group, where: † $P< 0.05$; and ††$P < 0.01$; ††† $P< 0.001$. *Symbol represents significance compared with the Pb-toxic group, where: *$P < 0.05$.

6. Effect of lead removal using *T. stipitatus* on gross morphology and relative organ weights in male albino rats

In rats of the Pb-toxic group (GII), gross morphology of the liver, lung, brain and prostate glands was slightly larger in size and paler in color; on the contrary, the testis was smaller in size and paler in color, compared to the normal control group (GI). On the other hand, these organs showed marked improvement in rats of the fungal biosorption treatment group (GIII), showing a normal size and color, as compared to normal control ones. Noticeable gross morphology of kidney, heart, spleen, epididymis and vesicula seminalis in the treatment group (GIII) appeared normal in color and size, as compared with the control group (GI).

The relative weights of the liver, lungs, brain and prostate glands were all significantly higher in the pb-toxic group (GII) compared to the untreated group (GI), but the kidney and accessory organs (with the exception of the testis) exhibited non-significant alterations (Fig. 7). The detection of an increase in relative organ weight may be the consequence of necrosis and apoptosis caused by Pb buildup in the affected organs **(Ibrahim *et al*., 2012)**. Heavy metal (Pb) absorption is substantially greater in intraperitoneal injection than in oral injection, which may be a contributing factor **(Ysart**

***et al.*, 2000; Ryan *et al.*, 2001; Wilhelm *et al.*, 2002)**. Since the rats in this research were given the heavy metal by oral administration, the effects on the aforementioned organs may be minor owing to intestinal absorption of the substance **(Iqbal *et al.*, 2021)**.

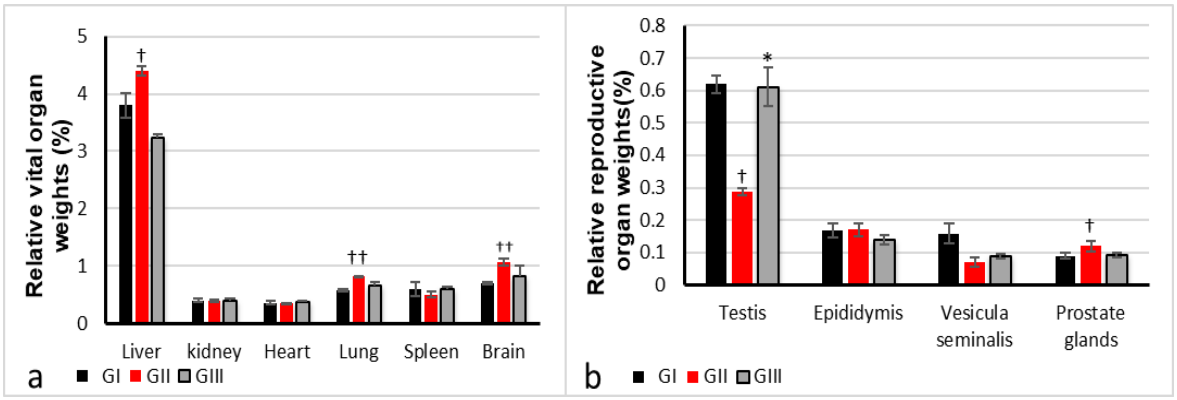

Fig. 7. Effect of lead removal using *T. stipitatus* on the relative organ weights of male albino rats. a) Relative vital organ weights. b) Relative reproductive organ weights. Data are expressed as mean ± SE. †Symbol represents significance compared with the negative normal control group, where: † $P< 0.05$ & †† $P < 0.001$. *Symbol represents significance compared with the Pb-toxic group, where: *$P < 0.05$.

7. Effect of lead removal using *T. stipitatus* on bioaccumulation in the liver and kidney tissues in male albino rats

Bioaccumulation of lead was measured in both liver and kidney tissues for the following reasons; 1) the liver due to its detoxification ability of toxicants (**Taha, 2022**) and its structure and functions can be changed according to the exposure period to metals toxicants. 2) the renal system is considered the primary system for the excretion of heavy metals and is a site of accumulation.

Table 3 demonstrates that normal control rats (GI) had little lead contamination in their livers but none in their kidneys. According to these findings, the presence of lead in the liver of the control rats that were intended to be free of metals suggests that the water and/or diet utilized were contaminated with Pb. Prevalance of Pb in the general environment may be to blame **(Tchounwou *et al.*, 2012)**. Lead bioaccumulation was significantly elevated in the liver and kidney tissues of the Pb-toxic group (GII), with bioaccumulation being greater in the liver than in the kidney tissue. These findings are consistent with those of **Bala *et al.* (2012)**, **Bala *et al.* (2013)**, and **Yuan *et al.* (2014)**, who also found that the liver contained more lead than the kidney. This disparity may be attributable to the liver's role in the detoxification of harmful compounds and the kidney's role as an excretory organ, which is responsible for the removal of toxins from the body through urine. Rats in the treatment group (GIII) revealed not detected lead amounts in liver and kidney tissues after bioremoval compared to rats before treatment. Lead was eliminated, seemingly as a result of a fungal biosorption treatment, according to these our results.

Table 3. Effect of lead removal using *T. stipitatus* on bioaccumulation in the liver and kidney of male albino rats.

Groups / Tissues	Lead concentrations (mg/kg)	
	Liver	Kidney
GI	0.24± 0.008	ND
GII	0.49± 0.051†	0.05 ±0††
GIII	ND	ND

Data are expressed as mean ± SE. †Symbol represents significance compared with the negative normal control group, where: † $P< 0.05$; and †† $P< 0.001$. ND: Not detectable.

8. Effect of lead removal using *T. stipitatus* on semen quality for male albino rats

Measuring the fertilizing potential of sperm was one way to get a sense of whether an animal was fertile or not, and both the number and moility of its sperm were crucial considerations **(Mahmoud *et al*., 2018; Taha & Soliman, 2019; Taha & Gouda, 2022)**. As can be shown in Figs. (8a, b), the Pb-toxic group (GII) had a markedly lower sperm count and sperm motility (progressive and non-progressive) than the untreated negative normal control ones (GI). In contrast, there was a statistically significant increase in the number of immotile sperm in the Pb-toxic group (GII), compared to the negative normal control group (GI). All the experimental groups' sperm morphology is depicted in Figs. (8c, d), and while there was a statistically significant increase in the total number of sperm abnormalities in the Pb-toxic group (before treatment) (GII) compared to untreated rats. Other researches **(Ronis *et al*., 1996; Sokol *et al*., 2002; Telisman *et al*., 2007)** have shown similar effects of Pb on the quality of sperm. The hypothesized cause for the Pb-toxic group's decreased sperm count, motility and normal sperm morphology is that Pb impeded the process of spermatogenesis, leading to the emergence of oligozoospermia. These findings strongly suggest that Pb has a detrimental influence on sperm maturation, leading to abnormal tail development **(Rani & Sinha, 2022)**. The possibility that lead may subsitue for zinc in metallothionein is also well-known **(Wirth & Mijal, 2010)**. Zinc aids in the maintenance of lipid bilayers in cell membranes. Zinc is also essential for spermatogenesis and the maturation of sperm via the conversion of histone to protamine **(Gatewood *et al*., 1990; Wang *et al*., 2019)**. Zinc deficiency may enhance the generation of sperm with abnormal morphology and disrupted chromatin structure **(Fallah *et al*., 2018; Wang *et al*., 2019)**, while lead deficiency can impede histone to protamine conversion. **Sen-Gupta *et al.* (2004)** and **El-Shahat *et al.* (2009)** suggested that an increase in oxidative stress byproducts and subsequent cellular death may be to blame for the observed rise in sperm abnormalities. Additional human research **(Hsu *et al*., 2009)** has linked elevated blood lead levels to an increase in sperm chromatin damage.

Both sperm count and motility improved significantly in the treatment group (GIII), whereas sperm abnormalities decreased significantly, returning to normal levels after being elevated in the Pb-toxic rats (GII) (Fig. 8). These results indicate that fungal biosorption treatment caused improvement in semen quality.

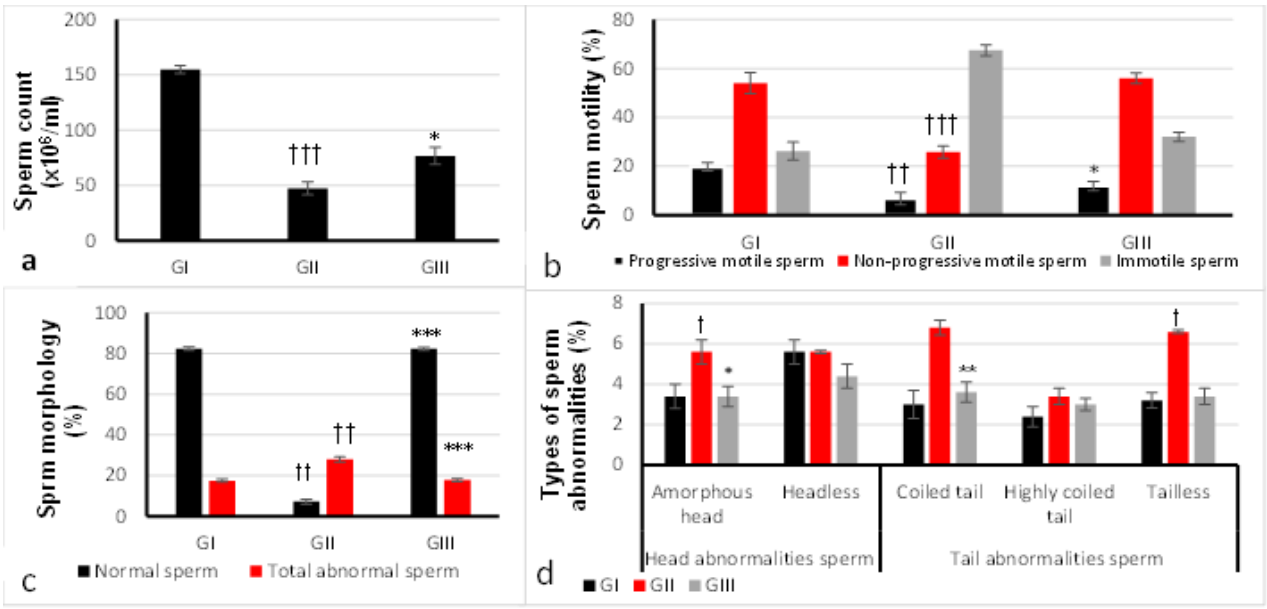

Fig. 8. Effect of lead removal using *T. stipitatus* on semen quality in male albino rats. (a) Sperm count, (b) Sperm motility, (c) Sperm morphology, and (d) Types of sperm abnormalities. Data are expressed as mean ± SE. †Symbol represents significance compared with the negative normal control group, where: † $P< 0.05$ and †† $P< 0.001$. *Symbol represents significance compared with Pb-toxic group, where: *$P< 0.05$; **: $P< 0.01$; and ***: $P< 0.001$.

9. Effect of lead removal using *T. stipitatus* on histopathological alterations in male albino rats

9.1. Liver

Liver sections from the negative control group were found to have a normal histological structure at a macroscopic level **(**Figs. 9a, b). Multifocal regions of mononuclear cells were randomly aggregated in the hepatic tissue, and enhanced portal fibroplasia were seen in the Pb-toxic group (GII) (Figs. 9c-g). Consistent with previous research **(Jarrar & Taib, 2012; Ibrahim *et al.*, 2014)**, these findings suggested that lead may interact with proteins and enzymes in the liver's interstitial tissue, disrupting the antioxidant defense mechanism and leading to reactive oxygen species (ROS) generation, which may mimic an inflammatory response and cause the appearance of inflammatory cells in the liver in those who have been exposed to lead **(Johar *et al.*, 2004)**. The treated group's livers showed normal on histology, with hepatocytes organized in cords and sinusoids unharmed (Figs.9. h-i). Lead biosorption by fungi seems to be responsible for the observed improvement in the treatment group.

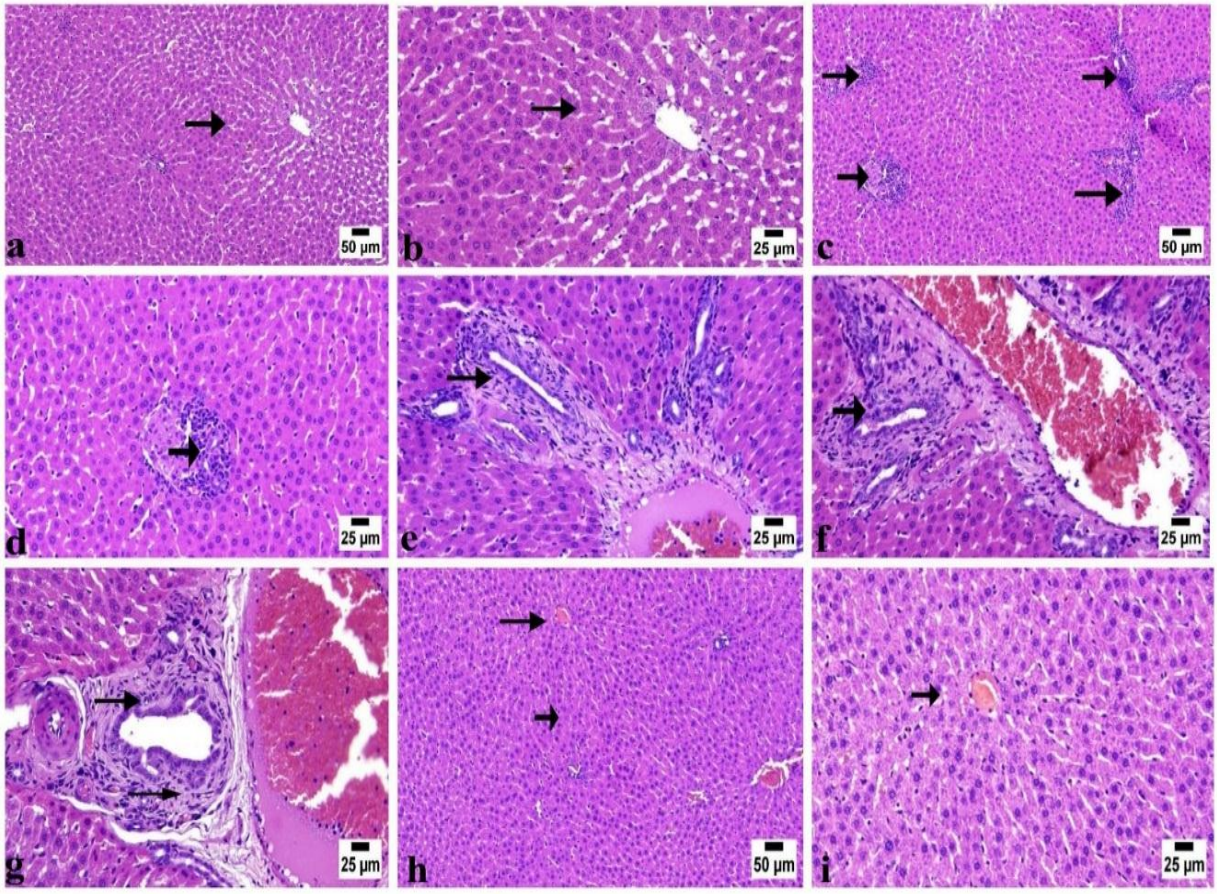

Fig. 9. Photomicrographs of liver tissue sections of male albino rats stained by (H & E). **(a&b)** The negative control group shows the normal histological structure of hepatic parenchyma (arrow). (c-g) The Pb-toxic group showed **(c)** multifocal aggregations of mononuclear inflammatory cells, **(d)** focal aggregations of mononuclear inflammatory cells, (**f&g)** portal fibroplasia with mononuclear inflammatory cell infiltration (arrows), and **(h-i)** The treatment group shows normal hepatic parenchyma (arrows).

9.2. Kidney

Histopathological analysis of the kidneys of untreated rats (GI) revealed no abnormalities in the cortex or medulla of the kidneys (Figs. 10a, b). Microscopic examination of kidneys of the Pb-toxic group (GII) showed multifocal perivascular edema and inflammatory cell infiltration associated with multifocal areas of interstitial nephritis in both the renal cortex and renal medulla. Cystically dilated renal tubules lined with attenuated epithelial cells were detected in the corticomedullary junction and renal cortex (Figs.10c-g). The heavy metal-induced degradation of glomerular infiltrations led to renal tubular degeneration, necrosis and fibrosis, which led to his kidney damage **(Uriu *et al*., 1998)**. These results coincide with those of **Ibrahim *et al.* (2014)**. Macroscopic analysis of the kidneys of the treatment group revealed significant enhancement, with normal renal cortex and renal medulla (Figs.10h-i). These improvement attributed to fungal biosorption of lead and indicate that the removal of Pb by using fungus *T. stipitatus* as biosorbent material doesn't cause any toxicity on the kidney of rats.

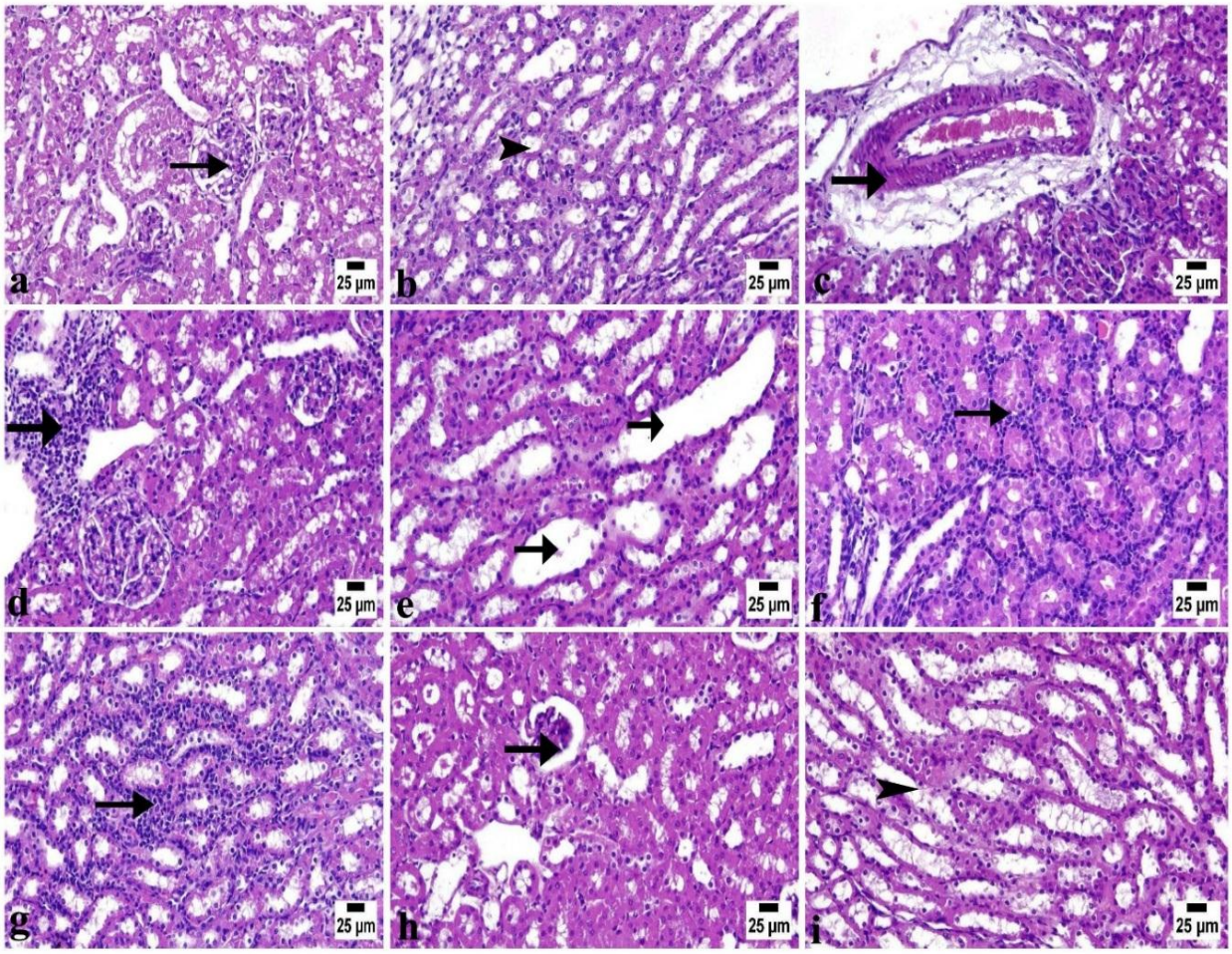

Fig. 10. Photomicrographs of kidney tissue sections of male albino rats stained by (H &E). **(a&b)** The negative control group shows normal renal cortex (arrow) and normal renal medulla (arrowhead). **(c-g)** The Pb-toxic group shows **(c)** perivascular edema and inflammatory cells infiltration (arrow), **(d)** focal interstitial nephritis with aggregation of mononuclear inflammatory cells (arrow), **(e)** cystically renal tubules lined with attenuated epithelial cells at the corticomedullary junction (arrow), **(f)** interstitial nephritis in the renal cortex (arrow), **(g)** interstitial nephritis in the renal medulla (arrow), and **(h-i)** The treatment group shows normal renal cortex (arrow) and normal renal medulla (arrowhead).

9.3. Testis

Microscopic examination of testes from the control group (GI) showed the normal testicular structure of seminiferous tubules that appeared packed by increased numbers of spermatogonial cells and Sertoli cells with the presence of numerous spermatids and numerous spermatozoa **(**Fig. 11 a). Testes of rats of Pb-toxic group (GII) showed several histopathological lesions including atrophied seminiferous tubules with a significant decrease in both diameter and epithelial height in addition to a significant reduction in stages of spermatogenesis (Johnsen's score), compared to normal ones (Figs. 11b-g & Table 4). Atrophied seminiferous tubules were characterized by irregular outlines,

testicular degeneration with exfoliation of the germinal epithelium into the lumen of seminiferous tubule and vacuolation of the germinal epithelium and presence of large congested blood vessels **(**Figs. 11b-g). These indicate the negative impact of lead that causes impairment of spermatogenesis. Such findings concur with those of previous reports **(El-Neweshy *et al*., 2013; Liu *et al*., 2016; Chen *et al*., 2018)**. This research confirms previous reports on the histopathological effects of lead exposure on the testes **(Elgawish & Abdelrazek, 2014; Bas & Kalener, 2016)**. Damage to Sertoli cells and the resulting disruption of cell connection is reflected in the exfoliation of germ cells into the tubular lumen **(Akhtar *et al*., 2009)**. Since the epithelial cells were sloughed into the lumen, the lumen shrank and the epithelial volume fraction decreased. Sertoli cell vacuolation and the near-total absence of spermatozoa are indicative of a dysfunctional spermatogenesis **(Creasy, 2001)**. Lead has been linked to the disruption of the blood-testis barrier because of its ability to substitute for calcium at zona adherens junctions. This might facilitate lead's entry and the deregulation of testicular tissue functions **(Wirth & Mijal, 2010)**. Increased adenosine synthesis, in line with hypoxia, led to vasodilation and increased blood flow in the Pb-toxic group (GII) testes with their dilated blood vessels and normalized oxygen levels **(Huether & McCance 2008; Mahmoud *et al*. 2018; Taha & Gouda 2022).**

Testicular examination of treatment group (GIII) showed normal seminiferous tubules that appeared packed by increased numbers of spermatogonial cells and Sertoli cells with the presence of numerous spermatids and numerous spermatozoa (Figs. 11h-i). In addition to the diameter and the epithelial height of seminiferous tubules, Johnsen's score showed a significant increase compared to the Pb-toxic group (GII) **(**Figs. 11h-i & Table 4). These indicated that lead removal by fungal biosorption revealed marked improvement in the structure of testes to retain to normal histology after treatment.

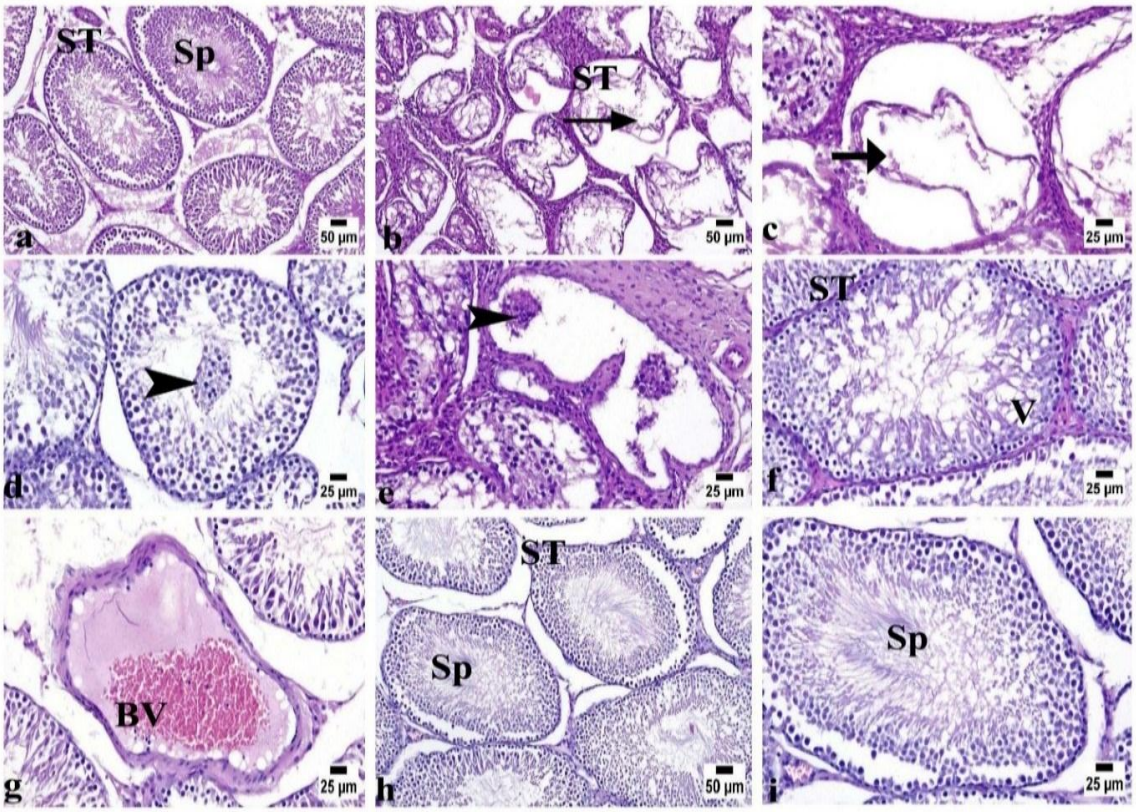

Fig. 11. Photomicrographs of testis tissue sections of male albino rats stained by (H & E). **(a)** The control group showed normal testicular structure consisting of stratified germinal epithelium and lumen filled with spermatozoa (Sp) many ovals or rounded seminiferous tubules (ST) with successful all stages of spermatogenesis including primary spermatocytes, spermatids, and a lumen filled with abundant spermatozoa (Sp). **(b-g)** The Pb-toxic group showing **(b&c)** atrophied seminiferous tubules (ST) with testicular degeneration and irregular outlines (arrow), **(d&e)** testicular degeneration with exfoliated of the germinal epithelium into the lumen of seminiferous tubule (arrowhead). **(f)** vacuolation of the germinal epithelium (V), **(g)** congestion of interstitial blood vessel (BV), (h-i) The treatment group shows normal seminiferous tubules (ST) containing numerous sperms (Sp).

Table 4. Effect of lead removal using *T. stipitatus* on morphometric analysis of testis and stages of spermatogenesis in male albino rats.

Groups	Testicular morphometric analysis (µm)		Stages of spermatogenesis
	Diameter	Epithelial height	Johnsen's Score (1-10)
GI	398.3±24.3	168.3±8.3	8.83±0.10
GII	92.5±10.7†	50±1.6†	3.47±0.28†
GIII	267.5±4.24*	131.19±4.68*	8.3±0.087*

Data are expressed as mean ± SE. †Symbol represents significance compared with the negative normal control group, where: † $P< 0.001$. * Symbol represents significance compared with the Pb-toxic group, where: *: $P< 0.001$.

9.4. Epididymis

Microscopic examination of the epididymis of untreated rats showed the normal histological structure of epididymis with epididymal ducts containing increased amounts of formed spermatozoa **(**Figs. 12a, b). Macroscopic examination of the epididymis of rats of the Pb-toxic group showing marked reduced spermatozoa content was accompanied by a significant reduction in the diameter of epididymal ducts (ductal atrophy) with marked atrophied duct hypospermia due to the reduced volume of sperm and fluid (Figs.12c, d & Table 5). These results are attributed to oxidative stress and lipid peroxidation, causing serious harm to the epididymis after Pb intoxication by increasing lipid peroxidation damage in the epididymis and causing differences in antioxidant levels in the epididymal sperm and the male reproductive system **(Latchoumycandane *et al*., 2003; Apaydin *et al*., 2021)**. In treatment group, macroscopic examination of the epididymis showed a significant increase in the diameter of epididymal ducts containing numerous mature spermatozoa inside epididymal ducts (Figs.12e, f & Table 5). These indicate that improvement took place after fungal biosorption of **ss**lead to retain to normal histological structure of epididymis.

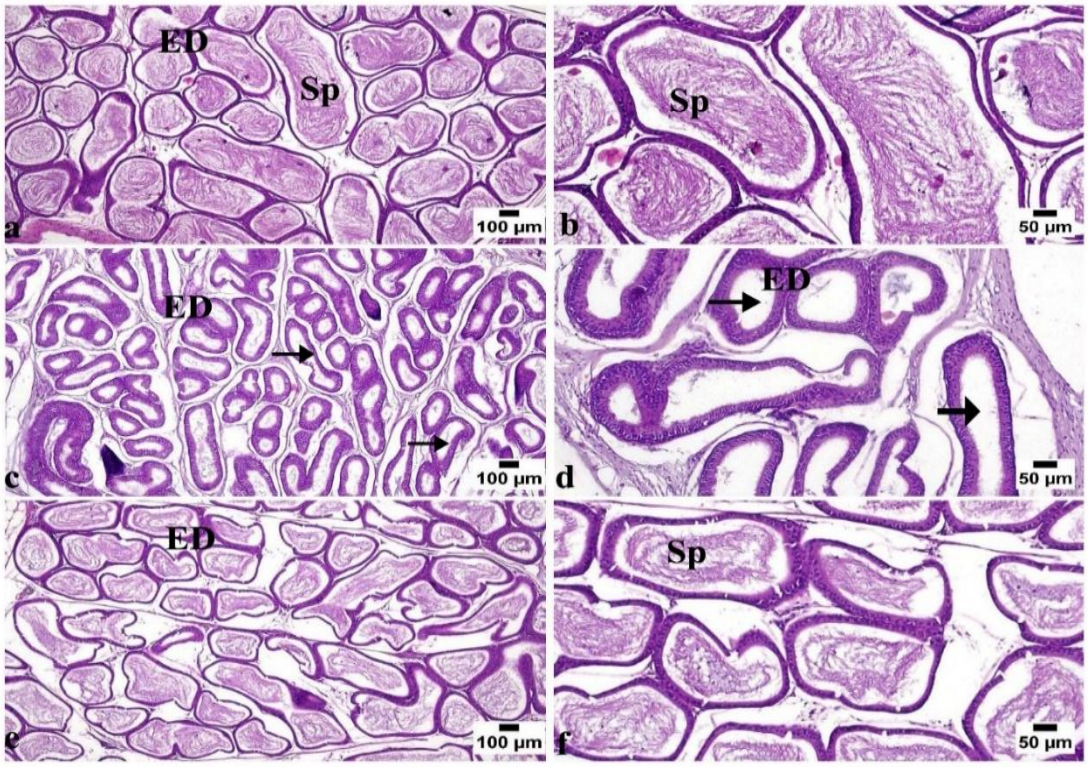

Fig. 12. Photomicrographs of epididymal tissue sections of albino rats stained by (H&E). (a &b) Normal control group showing epididymal tubules (ED) full of spermatozoa in the ductal lumen (Sp). (c &d) Pb-toxic group showing atrophy of epididymal ductal size (ED) marked with hypospermia, and (e & f) The treatment group showed normal epididymis with epididymal ducts containing numerous sperms (Sp).

Table 5. Effect of lead removal using *T. stipitatus* on diameter of the epididymal ducts of the epididymis in male albino rats.

Groups	Diameter of the epididymal ducts (µm)
GI	316.66±14.06
GII	91.66±5.45†
GIII	226±12.95*

Data are expressed as mean ± SE. †Symbol represents significance compared with the negative normal control group, where: † $P< 0.001$. *Symbol represents significance compared with the Pb-toxic group, where: *: $P< 0.001$.

CONCLUSION

The bioremoval of heavy metals from the environment is widely recognized as an economical, successful, and environmentally safe solution. Fungal dead biomass might be a low-cost way to remove harmful heavy metals from contaminated solutions via the biosorption method due to its special characteristics of metal binding. Our findings revealed that, under ideal circumstances, the dead biomass of the lead-resistant fungus *Talaromyces stipitatus* could biosorb lead ions with an efficiency of up to 96%. A dditionally, the treated aqueous solution did not cause any alterations in biochemical parameters, bioaccumulation of Pb in the liver and kidney or histopathological changes in various organs (liver, kidney, testis and epididymis). These data suggest *T. stipitatus* as a potential candidate for on-site cleanup of lead-contaminated areas, where it might contribute to the improvement of environmental conditions without endangering animals. Future studies should consider *T. stipitatus* for its biosorbent adsorption effectiveness in real wastewater.

Author Contribution Statement

Taha A. and Gouda SA: Conceived and designed the experiments; Taha A., Gouda SA, Mohamed S, Mahmoud MA, Saeed E, Fathy M and Mohamed N: performed the experiments; Taha A and Gouda SA: analyzed, interpreted the data, contributed materials, and wrote the paper equally. All authors have read and agreed to the published version of the manuscript.

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