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Biosorption of Cadmium from Polluted Waters Using Dead Biomass of the Fungus *Alternaria tenuissima* and its Toxicological Effects on Male Albino Rats

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ABSTRACT

It is important to find and identify more biological adsorbents that can effectively remove metals from water instead of using the traditional approach. In the present study, a fungal strain resistant to cadmium was isolated from polluted sites. It was identified morphologically and molecularly as *Alternaria tenuissima*, with an accession number of OQ691600. Heat inactivated biomass of *A. tenuissima* was evaluated for cadmium (Cd) biosorption. The biosorption process was conducted at a temperature of 28±2°C to remove Cd from water at an initial concentration of 92 mg/ L, under different conditions, such as pH (4–6), biosorbent dose (1–3 g/L), and sorption time (15–60 min). The obtained results demonstrated that the dead biomass of *A. tenuissima* effectively removed Cd, with maximum significant biosorption efficiency of 99.95±0.003% and capacity of 92.33±0.21 mg/g, which were achieved at a pH value of 6 for 30 min and a biosorbent dose of 1 g/L. Fourier transform-infrared (FT-IR) spectroscopy analysis revealed changes in the functional groups present on the surface of *A. tenuissima* biomass through the biosorption process. Following the biosorption process, three groups of male albino rats were used. The negative control group received only double-distilled water through oral administration. The Cd-polluted water group received a Cd aqueous solution (92 mg/L) through oral administration for 30 days. The treatment group received Cd aqueous solution (92 mg/L) through oral administration after treatment with *A. tenuissima* as the biosorbent material for a period of 30 days. The results showed that Cd caused a significant drop in weight gain and testicular index weight, as well as total protein, albumin, semen count, and motility. There were significant increases in abnormal sperm, the spleen, and brain index weight, as well as biochemical parameters, including aspartate aminotransferase, alanine aminotransferase, urea, creatinine, and uric acid, showed notable changes. The liver, kidney, testis, and epididymis of male albino rats showed several histopathological changes along with bioaccumulation of Cd traces in liver and kidney tissues. However, in the treatment group, all these findings were noticeably improved. Based on our findings, the dead biomass of *A. tenuissima* has the potential to be a very effective biosorbent for removing cadmium from water, without causing any hazardous effects on animals.

INTRODUCTION

Heavy metals, identified as the primary cause of water pollution, pose a global issue as highlighted by **Bahafid *et al.* (2017)** and **Taha *et al.* (2023a)**. Their significant negative impact on public health and the economy stems from their cumulative nature, a concern emphasized by **Andersson (1999)**. Cadmium (Cd) is known to be a toxic metal with serious effects on man, animals, and plants **(Pavlaki *et al*., 2016; Genchi *et al*., 2020**). According to **Nair *et al.* (2013)** and **Wijesekara *et al*. (2015)**, long-term exposure to Cd damages several body organs, including the liver, kidneys, heart, brain, and testicles, and also negatively affects semen quality. Moreover, the rate of Cd exposure increased over the past few decades as a result of increased human industrial activity, which increased the amount of Cd entering the environment **(Cuypers *et al*., 2010; Broerse *et al*., 2012; Dua *et al*., 2015)**.

The conventional methods for removing Cd include chemical precipitation, chemical oxidation or reduction, ion exchange, filtration, electrochemical treatment, reverse osmosis, membrane technologies, and evaporation; however, they can be pricey, ineffective, and even harmful to the environment (**Thaçi & Gashi, 2018**).

Biosorption is a promising method for the safe elimination of heavy metals from the environment using natural materials or wastes. Fungal biomass is one of the most attractive biosorbents currently in use **(Manguilimotan & Bitacura, 2018)**. The biosorption of Cd onto the surface of the fungal biomass (either living or dead) occurs through their binding ability to various functional groups. This process has many advantages, including small handling time, low energy, and chemical consumptions. Consequently, it was regarded as an environmentally friendly, cost-effective technology **(Fu & Wang, 2011; Saad, 2015; Alzahrani *et al*., 2017)**. Several fungal species were recognized as biosorbents to Cd, including *Aspergillus niger* **(Barros *et al*., 2003**), *Rhizopus cohnii* **(Luo *et al*., 2010)**, *T. tomentosum* and *T. asperellum* **(Mohsenzadeh & Shahrokhi, 2014),** in addition to *Aspergillus versicolor* **(Soleimani *et al*., 2016)**, *Penicillium aurantiogriseum* **(Bahobil *et al*., 2017)**, *Trichoderma harzianum*, *Alternaria alternata* **(Das *et al.*, 2019)**, and *Fennelia nivea* **(Aracagök *et al*., 2021)**.

Our prior research is deemed to be complete with the current study **(Taha *et al*., 2023b)**. Regarding our knowledge, no studies on cadmium biosorption using dead *Alternaria tenuissima* biomass have been conducted. Hence, the objective of this study was to employ the dead biomass of a cadmium-tolerant fungus, *A. tenuissima*, as a biosorbent for removing Cd from water. The research aimed to investigate the impact of different experimental factors, such as pH, contact time, and biosorbent dosage, which are parameters affecting biosorption, on the Cd biosorption capacity and efficiency. Additionally, we aimed to evaluate the impact of water treated with dead fungal biomass on general health, semen quality, organ weights, some biochemical parameters, Cd bioaccumulation in the liver and kidneys, and histological alterations in various organs (liver, kidney, testis, and epididymis).

MATERIALS AND METHODS

1. Chemicals and preparation of the stock solutions

Cadmium chloride ($CdCl_2$, 99%) was supplied from Alpha Chemika™ (Mumbai, India). For pH adjustment, sodium hydroxide (NaOH, 99%), and hydrochloric acid (HCl, 30-34%) were purchased from El-Nasr Pharmaceutical Chemicals Co., Adwic (Cairo, Egypt). Moreover, to create calibration curves for cadmium analysis, standard Cd solutions (Merck KGaA, Darmstadt, Germany) were used. Additionally, the reagent kits used to measure the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as well as total protein and albumin levels, were purchased from Spectrum, an Egyptian biotechnology company in the El Obour City industrial area of Cairo, Egypt. Furthermore, laboratory kits for the blood urea, creatinine, and uric acid were purchased from Biodiagnostic in Dokki, Giza, Egypt. Finally, cadmium chloride stock solution was prepared by dissolving weighed amount of $CdCl_2$ in double-distilled water. The solution was then diluted to the proper concentration and utilized immediately.

2. Isolation and morphological identification

Isolation of fungi from both polluted water at Ismailia canals and the soil surrounding it was accomplished using the serial dilution approach (**Iram *et al*., 2013**) and the spread plate method on potato dextrose agar (PDA) media supplemented with filter sterilized cadmium chloride at a concentration of 25 ppm. The cultures were then incubated at 28±2°C for a period of seven days, as described by **Joshi *et al*. (2011**). To prevent bacterial growth, streptomycin (35 $mgmL^{-1}$) was supplemented into the media. The dominant isolates were purified on PDA plates by the streak plate method, as outlined by **Bahobil *et al*. (2017)**. The fungus chosen for our study was selected based on its distinct morphological colonies and was coded as Alt2 until its identification. It was initially characterized based on macroscopic observation (pigmentation, shape, colony appearance, and texture), as well as microscopic observation (septation of mycelium, shape, and form), which were compared to **Ellis (1971)** descriptions. The morphological identification of the fungal isolate Alt2 was confirmed by genotypic-based identification.

3. Genotypic- based identification

Total genomic fungal DNA was extracted using a Quick-DNA™ Fungal Microprep Kit (Zymo Research) following the manufacturer's recommended manual methodology. Primers NL-1 (5'-GCATATCAATAAGCGGAGGAAAAG-3') and NL-4 (5'-GGTCCGTGTTTCAAGACGG-3') were used in polymerase chain reaction (PCR) to amplify the D1/D2 regions of the 28S rRNA gene (**Kwiatkowski *et al*., 2012; Taha *et al*., 2023b**). The PCR protocol consisted of an initial denaturation at 95 degrees Celsius for 2 minutes, followed by 35 cycles of denaturation at 95 degrees Celsius for 15 seconds, annealing at 50 degrees Celsius for 20 seconds, and extension at 72 degrees Celsius for 60 seconds. A last extension at 72 degrees Celsius for 5 minutes ended the amplification process. The PCR amplicons were sequenced at GATC Company, German, using ABI 3730x1 DNA analyzer. The sequence data of the isolated fungus was compared to other

related sequences using the nucleotide BLAST search tool (blast.ncbi.nlm.nih.gov/Blast.cgi) of the National Center for Biotechnology Information (NCBI) and submitted into the NCBI GenBank database with an accession number. The neighbor joining (NJ) method with one thousand bootstrap replications was used to create phylogeny dendrograms in MEGA 11 software version 11 (**Felsenstein, 1985; Tamura *et al*., 2021**).

4. Tolerance test

Further screening of *Alternaria tenuissima* Alt2 at varying cadmium (Cd) concentrations (25, 50, 100, 200, and 400 ppm) was studied to determine its tolerance index (TI). Disks of the tested fungus, 7 mm in diameter, were inoculated into the middle of PDA control plates (without Cd) and test PDA plates (Cd supplemented), then incubated at 28±2°C. Mycelial radial growth was observed and recorded after seven days of incubation. The tolerance index (TI) was determined by dividing the fungal growth in the presence of metals by the fungal growth on the control plate (which did not contain heavy metals) over the same period of time **(Akhtar *et al*., 2013)**. The following categories of fungal heavy metal resistance were established according to the method of **Văcar *et al*. (2021)** using TIs: sensitive at 0, very low resistance (0 > TI > 0.29), low resistance (0.30 > TI > 0.49), moderate resistance (0.50 > TI > 0.69), high resistance (0.70 > TI > 0.89), and very high resistance (> 0.90).

5. Culture conditions and fungal biosorbent preparation

Ten-day-old spores of fungal PDA culture were inoculated into potato dextrose broth media (PDB) under shaking conditions (125 rpm) at 28±2°C for 7 days. Biomass was then filtered using filter paper, rinsed with double-distilled water to remove non-biomass particles, and autoclaved at 121 degrees Celsius for 15 minutes before being oven dried. The dry, dead fungal biomass was blended into powder using a mortar and pestle to be used in the biosorption process **(Luo *et al*., 2010)**.

6. Factors affecting cadmium biosorption and cadmium analysis

To check the impact of environmental parameters on biosorption process, the cadmium biosorption capacity and efficiency utilizing fungal biosorbent were studied at various pH, sorption time, and biomass dosage. All tests were done in 100 mL cadmium solution (92 mg/L) at a temperature of 28±2°C with a stirring speed of 125 rpm. The pH was adjusted to 4, 5, and 6 to determine the impact of initial pH on cadmium biosorption by adding 0.1N NaOH and 0.1N HCl to the reaction mixture. The optimal sorption time for cadmium biosorption was determined by analyzing the concentration of Cd in the solution mixture at regular times (15, 30, and 60 mins). The effect of biomass dosage was also determined by altering its dose from 1 to 3 g/L. The estimation of residual concentration of cadmium (biomass free supernatant collected after filtration) in each parameter at optimized study was carried out using Savant AA; GBC Scientific Equipment atomic absorption spectrometer in the flame module. Each experiment was conducted in triplicate, and the final results were compared to the metal concentration at the start of the experiments using statistical analysis. Equations 1 and 2 were used to

determine the biosorption capacity (q) and efficiency (E) of dead biomass for Cd, as outlined by **Fan *et al*. (2008)** and **Manguilimotan and Bitacura (2018)**, as follows:

$$q = \frac{V\,(C_i - C_f)}{m} \qquad (1)$$

$$E = \frac{C_i - C_f}{C_i} \times 100 \qquad (2)$$

where:

q is the biosorption capacity (mg/g)

C_i is the initial concentration of Cd ions (mg/L)

C_f is the final concentration of Cd ions (mg/L)

m is the biosorbent dose (g)

V is the volume of metal solution (L).

E is the biosorption efficiency (%)

7. Analysis of fungal biosorbent by FT- IR spectroscopy

The major functional groups that are present on the surface of *Alternaria tenuissima* dead biomass and responsible for Cd adsorption were determined using Fourier transform-infrared (FT-IR) analysis. The spectra of biomass before biosorption (control) and after biosorption (Cd-treated) were compared using an FT-IR spectrometer (Alpha II; Bruker, Massachusetts, USA) throughout a wave number range of 400 to 4000 cm^{-1}. To reduce the potential influence of OH^- on the fungal surface, both control and Cd-treated dead biomasses were dried overnight before analysis.

8. Animals and experimental design

The National Research Center provided fifteen adult male Wistar albino rats (*Rattus norvegicus*, 108±2g) (Cairo, Egypt). During the experiment, the rats had unrestricted access to conventional rodent chow and tap water. They were kept in proper cages at the Zoology Department’s Animal House in the Faculty of Science for 10 days prior to the start of the experiment to acclimate to the laboratory conditions. This included exposure to natural light and dark cycles, a temperature of 22 degrees Celsius, and humidity ranging from 40–60 percent. The Research Ethics Committee of Ain Shams University approved this study (ASU-SCI/MICR/2023/8/1), and we made every attempt to lessen the suffering of the rats. Consequently, only a small number of animals were used to ensure valid scientific findings.

For each of the three groups, five rats were randomly selected. Treatments were categorized as follows:

Group (A): Animals were orally given 1 mL of double distilled water only and served as the negative control for 30 days.

Group (B): Animals were orally given double distilled water with Cd- chloride (92 mg Cd/L) and kept as the positive control for 30 days (Cd-polluted water group).

Group (C): Animals were orally given 1 mL of double distilled water with Cd-chloride (92 mg Cd/L) for 30 days after being treated with *Alternaria tenuissima* (dead fungal biomass) as a biosorbent material (fungal treatment group).

Throughout the entire experimental study, all rats were observed once per day for clinical toxicity and twice for mortality.

9. Sample collection

After collecting blood samples through cardiac puncture and allowing them to clot, the specimens were centrifuged at 1,800×*g* for 10 minutes. The serum supernatants were stored at –20°C for liver and kidney function testing until biochemical parameters could be tested. The necropsy included the removal and dissection of the liver and kidneys. Both pieces were preserved in 10% formalin for histological examination, and one was stored at –20°C for the investigation of trace element residues. Histological assessment of the right testis and epididymis was conducted. Moreover, the left epididymis was placed in 2 mL of saline (NaCl 0.9 percent) at 35 degrees Celsius for further semen analysis.

10. Evaluation of body weight change and organ coefficient

By using chloroform, rats were anesthetized. Prior to the experiment and again just before dissection, all rats were weighed using an automated balance to determine their initial and final weights, respectively. The percentage of the change in body weight was then calculated using the following formula:

Percentage of the change in body weight = [(initial weight-final weight)/initial weight] × 100

The following organs were removed; the testis, epididymis, liver, kidney, heart, lung, spleen, brain, seminal vesicles, and prostate gland. These organs were then cleaned of any remaining blood and dried before being weighed. To obtain the organ coefficients, the formula (the organ weight/ final body weight × 100) was used.

11. Biochemical analysis

The activities of aspartate aminotransferases (AST) and alanine aminotransferase (ALT) enzymes were measured in accordance with **Bergmeyer *et al.* (1978)**. Total serum protein was evaluated by using the method of **Yatzidis (1987)**. Additionally, the **Doumas *et al.* (1971)** method was used to measure levels of serum albumin. To assess kidney function, serum levels of urea, creatinine, and uric acid levels were tested. According to **Henry *et al.* (1974)** and **Patton & Crouch (1977)**, colorimetric methods were used to measure the concentrations of creatinine and urea, respectively. The technique described by **Whitehead *et al.* (1991)** was used to measure uric acid in the blood.

12. Analysis of trace cadmium residue in liver and kidney tissues

After conducting the necropsies of rats, their liver and kidney tissues were removed and stored as frozen samples before being sent to the lab for the analysis of heavy metal concentrations. Prior to analysis, the samples underwent digestion using a CEM Microwave Sample Preparation System (MDS-2000, USA). Nitric acid (HNO_3) was added to containers containing one gram of powdered material. The containers were left

overnight to ensure an adequate response. Once placed on the system's rotation, a heating program was initiated and allowed to run until the digestion process was complete. After 5 minutes, the samples were allowed to cool, and the turntable was removed from the system. For cadmium determination, 25 mL of distilled water was combined with the digested samples and then analyzed in a Flame Atomic Absorption Spectrophotometer (Savant AA, GBC Scientific Equipment) following the outlined guidelines of the American Public Health Association **(APHA-3111B, 2017)**. The concentration of each cadmium was determined at a specific wavelength and slit width. The resulting concentration in mg/kg of tissue was calculated using the following formula:

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

13. Evaluation of semen parameters

Sperm motility was evaluated using a 400x light microscope 15 minutes after the left epididymis was removed and sliced with tiny sterile scissors and placed in a petri dish containing 2 mL of physiological saline at 35°C. According to **Taha *et al*. (2023b**), sperm motility is classified into three distinct types: progressive motile, non-progressive motile, and immotile. The sperm count was determined by infusing a sample of sperm suspended in saline at 37ºC into a Neubauer type, hemocytometer. Five independent counts were taken and averaged. Each sample from each rat was counted at least five times to ensure accuracy. The data were recorded as the number of sperm per milliliter. Smeared sperm on microscope slides were dried overnight before being preserved in methanol. After fixation, samples were stained for an hour with 1 percent (w/v) aqueous eosin Y, washed with distilled water, dehydrated, cleared, and then mounted in neutral glue under a coverslip. Using a light microscope with a 1000x oil immersion objective lens, 100 spermatozoa from each sample were examined (**Taha & Soliman, 2019)**. Abnormalities in sperm percentage were expressed as a percentage of the spermatozoa counted. The sperm was categorized as abnormal according to **WHO (2000)**.

14. Gross and histological examination

For the sacrificed male albino rats, gross tissue examinations were conducted to evaluate any alterations. Samples of the liver, kidney, testis, and epididymis from each rat were weighed and then fixed in 10% formalin; they were subsequently dried in a succession of progressively more astringent ethanol, cleaned in terpineol, and finally embedded with paraffin. Following deparaffinization in xylol, 5µm thick slices were stained with hematoxylin and eosin (H & E). The slides were then covered with a synthetic resin mounting media (DPX) consisting of Di styrene, a plasticizer, and xylene, and mounted under a microscope **(Mahmoud *et al*., 2018; Taha *et al*. 2023b)**. A light microscope was used to examine the slides.

15. Morphometric measurements for male reproductive organs and Johne's score

At a magnification of 400×, ten randomly chosen fields from the testis and epididymis were examined to determine the average tubule diameter and epithelium height of seminiferous tubules, as well as the diameters of epididymal ducts. We used Johnsen's

scoring system to assess phases of spermatogenesis. Each tubule is given a score between 10 and 1 based on whether or not it contains the main cell types, indicating its level of development. Using the criteria established by **Mizuno *et al*. (2007)**, we can classify spermatogenesis, as shown in Table (1). Selecting 10 seminiferous tubules/ rats at random yields the inferred rating.

Table 1. Classification of spermatogenesis stages

Score	Indication meaning
10	Perfect tubules and complete spermatogenesis
9	A large number of spermatozoa and disorderly spermatogenesis
8	A small number of spermatozoa
7	The presence of numerous spermatids but no spermatozoa
6	The presence of few spermatocytes
5	No spermatozoa or spermatids but many spermatocytes present
4	The presence of few spermatocytes
3	The presence of only spermatogonia
2	The presence of no germ cells but of Sertoli cells
1	The absence of both germ cells and Sertoli cells

16. Statistical analysis

The one-way analysis of variance (ANOVA) and Tukey's *post hoc* multiple comparisons test were used to statistically analyze the data by Minitab V17 software. The difference was significant if the *P*-value was less than 0.05. The results were displayed as mean± standard error (SE).

RESULTS AND DISCUSSION

1. Fungal identification

One of the most promising methods for environmental cleanup is the biosorption of heavy metals by microorganisms **(Konopka *et al*., 1999)**. A wide variety of fungi, including *Aspergillus*, *Penicillium*, *Alternaria*, *Geotrichum*, *Fusarium*, *Rhizopus*, *Monilia*, and *Trichoderma* were present in the soil polluted with heavy metals such as cadmium, nickel, copper, chromium, and cobalt (**Zafar *et al*., 2007)**. Moreover, the presence of fungal species in sewage sludge water plants, heavy metal polluted freshwater habitats, and sewage and industrial wastewaters was reported by **López and Vázquez (2003)**, **Iskandar *et al*. (2011)** and **Iram *et al*. (2013)**. The fungal isolate Alt2 was chosen for this study due to its frequency and distinctive characteristics, and it was preliminary identified as a member of genus *Alternaria* according to its macroscopic and microscopic morphology on PDA medium. It was observed as greyish green cottony growth with a dark reverse. The conidia were typically ovoid or obclavate, smooth-walled, formed a linear chain, and included both transverse and longitudinal septa, as shown in Fig. 1a, b. The morphological identification was confirmed by molecular identification. Based on

morphological and molecular characteristics, the fungal isolate Alt2 was identified as *Alternaria tenuissima* and was assigned to GenBank with accession number OQ691600. In the current study, primers targeting the rDNA large subunit D1/D2 regions were used for molecular identification of fungi due to their specificity and sufficient length compared to ITS regions **(Young *et al.*, 2022)**. The phylogenetic tree representing the sequences data from NCBI of the closest related species to *Alternaria tenuissima* Alt2 sequence was constructed using the neighbor joining method (Fig. 1c).

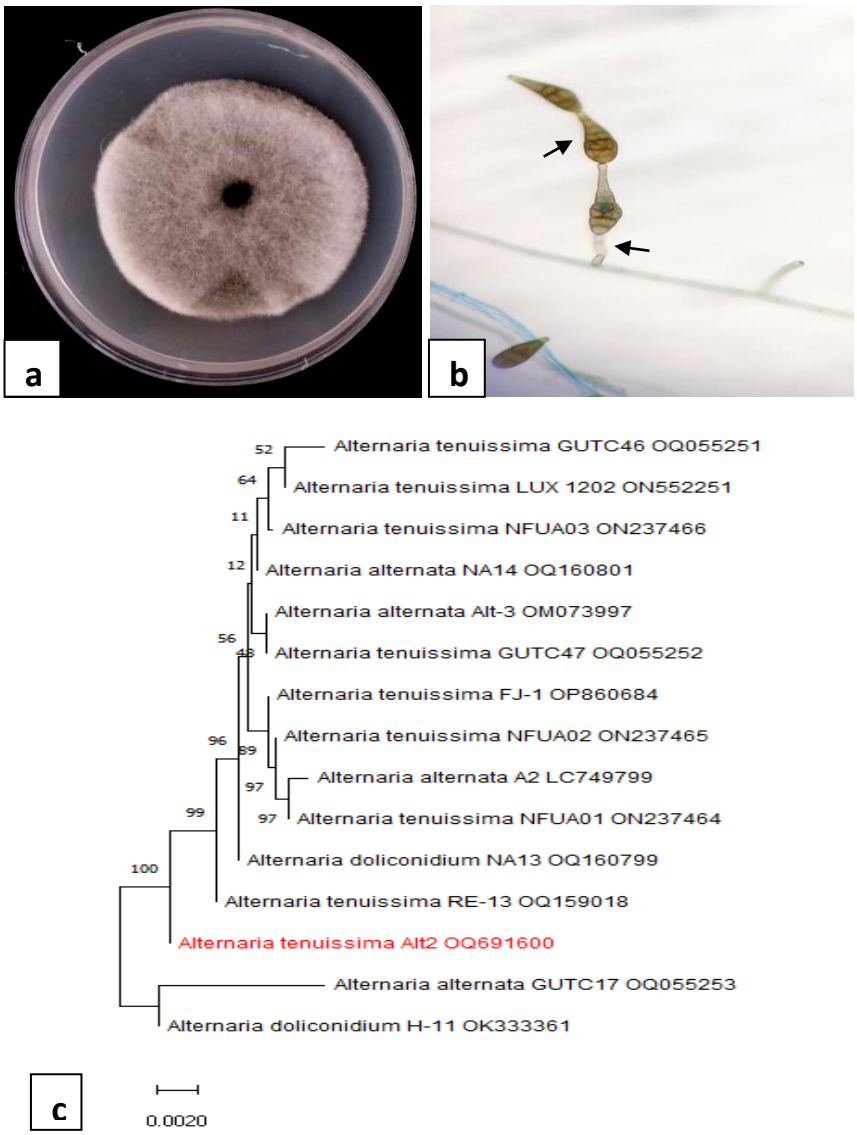

Fig. 1. Morphological and molecular identification of *Alternaria tenuissima* isolate Alt2 showing: (a) Colony growth on potato dextrose agar (PDA) media at 28±2°C for 7 days, (b) Conidiophore and conidia chain (arrows) shown at a magnification of 400×, and (C) Neighbor-joining tree based on the D1/D2 region sequences showing the relationship of *A. tenuissima* Alt2 (OQ691600) in red color and other related gene sequences. Bootstrap values were obtained from 1000 replicates

2. Cadmium tolerance test

The tolerance level of fungi can be determined through a tolerance index (TI) assay. It is important for researchers to find fungi that can grow and multiply in the presence of high amounts of heavy metals. According to this, the fungi can establish colonies and adapt to a polluted environment, the better for the biosorption process **(Fazli *et al*., 2015)**.

In cadmium enriched media, *Alternaria tenuissima* radial growth was significantly different from the control, and tolerance index calculations of *A. tenuissima* in comparison to its controls, using an average of the mycelial growth of three replicates revealed a very high resistance (TI, 0.91) at 25 ppm and moderate resistance to 50 ppm (TI, 0.53). However, *A. tenuissima* indicated low resistance and very low resistance at 100-200 ppm, with tolerance indexes of 0.38- 0.28, respectively, as shown in Fig. 2. The low tolerance index reveals the growth-inhibiting effect of heavy metals **(Ge *et al*., 2011)**. The differing resistance levels of fungi to heavy metals are directly related to the biological function of the strain **(Fazli *et al*., 2015)**. The severe inhibition of fungal growth at a Cd concentration of 400 ppm was attributed to the inhibitory effect of higher cadmium concentration on *A. tenuissima* growth. Similar to our observations, **Malik (2004)** reported that high concentrations of heavy metals are harmful to fungal and bacterial development. According to previous reports, a variety of fungal species from the genera *Alternaria, Aspergillus, penicillium, Trichoderma* and *Fusarium* showed different tolerance for cadmium at different concentrations that related directly to the biological function of the strain **(Joshi *et al*., 2011; Fazli, *et al*., 2015; Bahobil *et al*., 2017; Hassan *et al*., 2021)**.

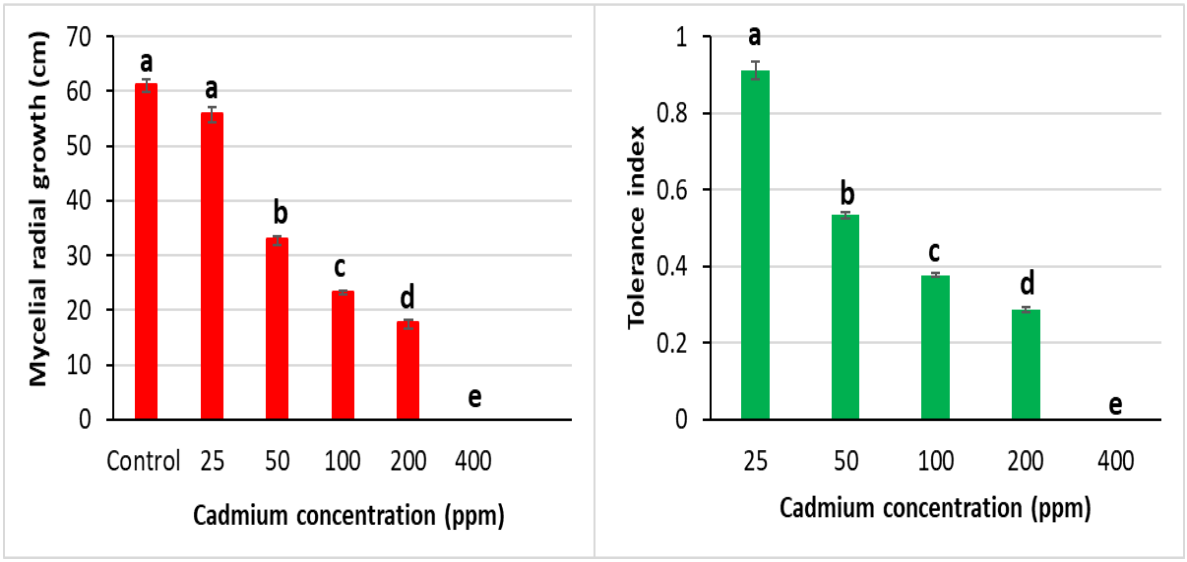

Fig. 2. Effect of varied concentrations of cadmium (Cd) on: (a) Mycelial radial growth, and (b) Tolerance index levels of *Alternaria tenuissima* after 7 days of incubation. The means (n = 3) ± SE (the line on each bar) are used to express values. The different letters represented on bars indicated significant difference ($P< 0.05$) according to Tukey's multiple comparison test

3. Factors affecting cadmium biosorption

The feasibility of Cd biosorption from water using dead fungal biomass (*Alternaria tenuissima* Alt2 OQ691600) was examined in the current investigation under different parameters such as pH, sorption time and biomass dosage. The cell wall of non-viable cells plays a crucial role in absorbing metals. Our research included autoclaving, drying, and grinding fungal biomass for use in biosorption study. **Lopez and Vázquez (2003)** reported that heat, chemicals, and grinding increase the accessible surface area and the number of surface binding sites, which improve metal biosorption.

3.1. Effect of initial pH

In biosorption process, pH has an impact on the solubility of metal ions and the overall charge of the adsorbent, which affects the electrostatic binding of ions to matching functional groups **(Mohsenzadeh & Shahrokhi, 2014)**. The findings of Cd biosorption using *A. tenuissima* dead biomass at pH values of 4, 5, and 6 are shown in Fig. 3. The findings postulated that at a pH value of 6, the biosorption capacity and efficiency of Cd were both at their highest values of 27.05±0.06 mg/g and 87.8±0.05 percent, respectively, whereas at a pH of 4, the biosorption capacity was 14.127±0.30 mg/g and the efficiency was 45.88±0.89 percent. **Gouda and Taha (2023)** reported that pH is altering the sorbents active binding sites (such as hydroxyl, carboxyl, sulfhydryl, sulfonate, and phosphonate) to facilitate the heavy metals biosorption. Our findings attributed to the degree of ionization of functional groups present on the biomass surface which rises at pH near to neutral resulting in enhanced biosorption of Cd and other metal cations, including Al, Pb, Co, Mn, Ni, and Zn. On the other hand, the competing biosorption processes between H^+ and Cd ion may be responsible for reducing adsorption efficiency at low pH **(Zhao *et al.*, 2011)**. In order to prevent cadmium from precipitating as hydroxide, higher pH values were not included in our study **(Gouda & Taha, 2023)**.

Similar to our results, **Hassan and El-Kassas (2011)** found that cadmium absorption by marine fungus *Aspergillus cristatus* increased from a pH value of 3 to 6, with maximal uptake occurring at a pH value of 6. Maximum cadmium adsorption capability by *Alternaria alternata* and *Penicillium aurantiogriseum* was found to occur at a pH value of 6, as shown by the research of **Bahobil *et al.* (2017)**. However, **Shakya *et al.* (2015)** and **Verma *et al.* (2016**) reported that the metal ions were removed at a higher rate from a pH range of 5-8.6 by number of fungal species, including *Penicillium resedanum*, *Aspergillus wentii*, *Eupenicillium katangense,* and *Alternaria alternata*.

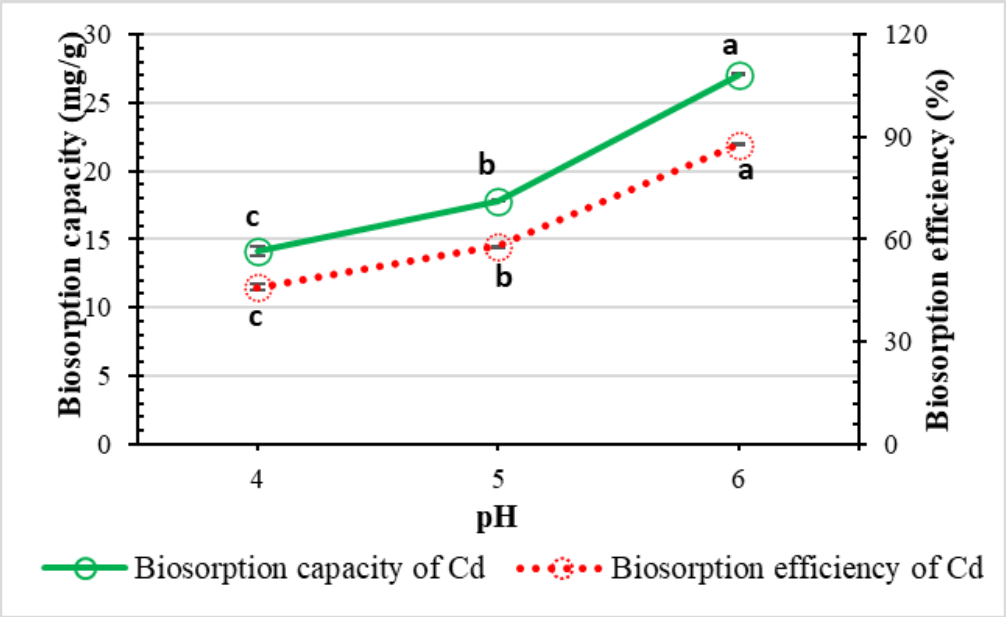

Fig. 3. Effect of initial pH on the biosorption capacity and efficiency of Cd. Biosorption conditions: Temperature (T) = 28°C; initial metal concentration (C_i) = 92 mg/L, sorption time (t) = 30 min, and biosorbent dose (D) = 3 g/L. The means (n = 3) ± SE are used to express values. The different letters indicated significant difference ($P< 0.05$) according to Tukey's multiple comparison test

3.2. Effect of sorption time

The biosorption experiment was performed at 15, 30, and 60 minutes with a pH of 6. The biosorption capacity and efficiency of Cd changed significantly at longer sorption times, as shown in Fig. 4. Our finding observed that the cadmium biosorption capacity using *A. tenuissima* at an sorption time of 30 min reached the highest significant value (27.05±0.081 mg/g) with biosorption efficiency of 87.95±0.16%, whereas adsorption of Cd decreased after 30 minutes. Our results are consistent with those of **Vimala and Das (2009)** and **Thapar (2022)**, who found that a greater number of active sites on the biomass led to a higher biosorption rate for heavy metals early in the biosorption process; however, subsequent biosorption decreased over time due to an attachment-controlled process due to the lack of sorption sites available. **Hassan *et al.* (2018)** also found that *Neopestalotiopsis clavispora* KY624416 was able to biosorb Cd metal and absorb it within 30 minutes. According to **Renu *et al.* (2016)**, cadmium removal is most effective when the contact period is between 5 and 120 minutes. In addition, **Ali *et al.* (2021)** discovered that increasing the exposure duration to 30 minutes sped up the biosorption of Cd utilizing heat inactivated *Penicillium chrysogenum* and *Cephalotheca foveolate*. Contrastingly, **El-Sayed and Reda (2011)** and **Soleimani *et al.* (2016)** reported an abrupt increase in cadmium biosorption after 30 minutes, reaching its peak at 60 minutes, and then gradually decreasing after 90 minutes of exposure. This phenomenon was observed using *Cunninghamella elagans* and dead *Aspergillus Versicolor*, respectively.

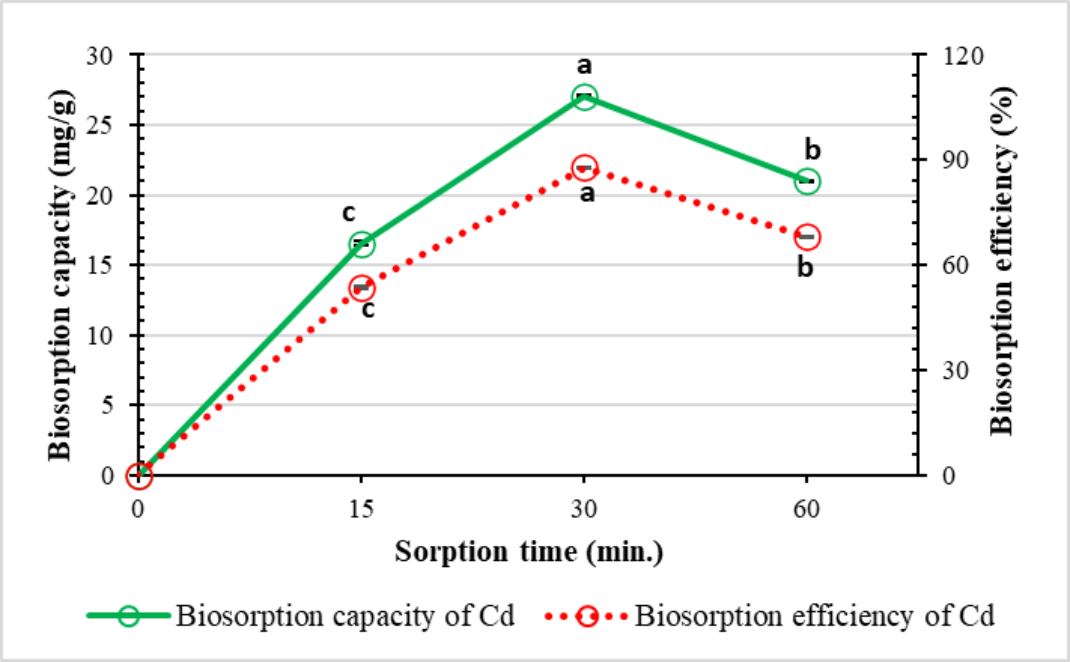

Fig. 4. Effect of sorption time on the biosorption capacity and efficiency of Cd using *A. tenuissima* dead biomass. Biosorption conditions: T = 28°C; pH = 6; C_i = 92 mg/L; and D = 3 g/L. The means (n = 3) ± SE are used to express values. The different letters indicated significant difference ($P < 0.05$) according to Tukey's multiple comparison test

3.3. Effect of biosorbent dosage

One of the key factors for efficient cadmium biosorption is the amount of biosorbent used. Our findings revealed that the maximum significant cadmium biosorption capacity and efficiency were 92.33±0.21 mg/g and 99.95±0.003%, respectively, with 1 g/L of *A. tenuissima*. However, the efficiency decreased to 87.94±0.17% with increasing biosorbent dose to 3 g/L (Fig. 5). It is clear from our observations that the Cd biosorption was significantly influenced by the dosage of the biosorbent. This is related to the correlation between the biosorbent dosage added to the solution and the number of binding sites accessible for adsorption of the metal. The biosorbent dose-dependence of metal adsorption suggests that a larger amount of metal is adsorbed when the distance between particles is sufficient. However, this adosrption decreases with an increase in biomass dosage. This phenomenon may be attributed to electrostatic interactions between biomass particles, leading to the aggregation of cells, hence reducing the number of accessible binding sites **(Cho *et al.*, 2010; Aracagök *et al.*, 2021)**.

Our results are corroborated with the findings showing that the biosorption capacity of heavy metals (cadmium, zinc, and lead) decreases with increasing the biomass of *Penicillium simplicissimum*, *Aspergillus niger*, and *Fennelia nivea* **(Júnior *et al.*, 2003; Fan *et al.*, 2008; Aracagök *et al.*, 2021)**. In addition, **Luo *et al.* (2010)** found that at initial different concentration of cadmium, the absorption of Cd by *Rhizopus cohnii*

reduced with increasing doses. Our results are in contrast to those of **José *et al.* (2019)**, who claimed that increasing the quantity of biomass enhances metal removal. **Javaid *et al.* (2011)** reported that when the amount of biomass from *Pleurotus ostreatus* grows, the amount of metal ions removed also increases. This phenomenon happens because the number of binding sites accessible for metal is proportional to the amount of the additional biosorbent.

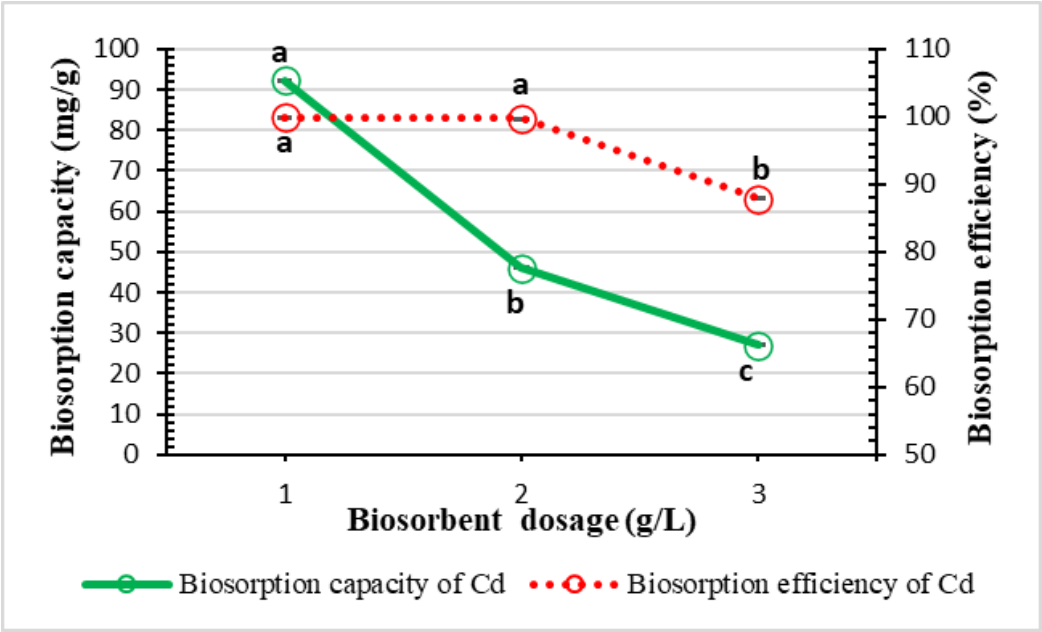

Fig. 5. Effect of biosorbent dosage of *A. tenuissima* dead biomass on the biosorption capacity and efficiency of Cd. Biosorption conditions: T = 28°C; pH = 6; C_i = 92 mg/L and t = 30 min. The means (n = 3) ± SE are used to express values. The different letters indicated significant difference (P< 0.05) according to Tukey's multiple comparison test

4. FT-IR analysis of fungal biosorbent

Metal ions may be biosorbed by the microbial cell wall surfaces, which is an impressive interaction mechanism that is characterized by Fourier transform-infrared spectroscopy (FT-IR) **(Sundararaju *et al.*, 2020)**. Numerous surface complexations models have been used to represent the metal adsorption by microorganisms in relation to the potential functional groups (nature of the potential cell-metal interaction) **(El-Sayed & El-Sayed, 2020)**. According to earlier studies, metal ions are adsorbed to the surface of cells through interaction with metal-functional groups like carboxyl, phosphate, hydroxyl, amino, sulphur, sulphide, and thiol (**Wang & Chen, 2006; Gouda & Taha, 2023)**. The FT-IR spectra of *Alternaria tenussemia* biomass before biosorption (control) and after biosorption (Cd-treated) are represented in Fig. 6 and Table 2. After Cd treatment, the hydroxyl and amine peaks in the FT-IR spectrum of biomass moving from 3289 to 3285 cm^{-1} owing to the stretching bond of the N-H from the amino group and the bounded hydroxyl group. Peaks reflecting the C-CH_3 stretching vibration, C=O stretching, N-H

bending, and weak aromatic bands shifted from 2923 to 2921 cm^{-1}, from 1623 to 1625 cm^{-1}, from 1553 to 1551 cm^{-1}, and from 1456 to 1452 cm^{-1}, respectively. The shift of the peak from 1238 to 1240 cm^{-1} indicates the coupling of the stretching band of C–N and the bending band of N–H from amide III. The aforementioned FT-IR spectra allowed us to determine that the -OH, -NH, -C=O, -C-H, and C-N functional groups were the most important for Cd biosorption on the surface of the dried dead biomass of *Alternaria tenuissima*. Our results are consistent with the results of **Mukhopadhyay (2008)**, **El-Sayed (2014)**, **Xia *et al.* (2015)**, and **Sundararaju *et al*. (2020)**.

Table 2. FT- IR analysis *Alternaria tenuissima* dead biomass before and after Cd biosorption

Peak	Before Cd biosorption (control)	After Cd biosorption (Cd- treated)	Functional groups
1	3289	3285	Stretching bond of the N–H from amino group and bounded hydroxyl group.
2	2923	2921	–CH stretching vibration of C-CH_3
3	2853	2853	C–H stretching
4	1744.9	1744.9	COO– stretching
5	1623	1625	C=O stretching
6	1553	1551	N–H bending
7	1456	1452	Weak aromatic bands
8	1370	1370	Symmetrical stretching band of carboxyl
9	1238	1240	Coupling of the stretching band of C–N and the bending band of N–H from amide III
10	1151.5	1151.5	The amino group C-N
11	1021.8	1021.8	C–O stretching of sugar alcohol
12	570	570	ν (P–O) + ring deformation, and ν C-S

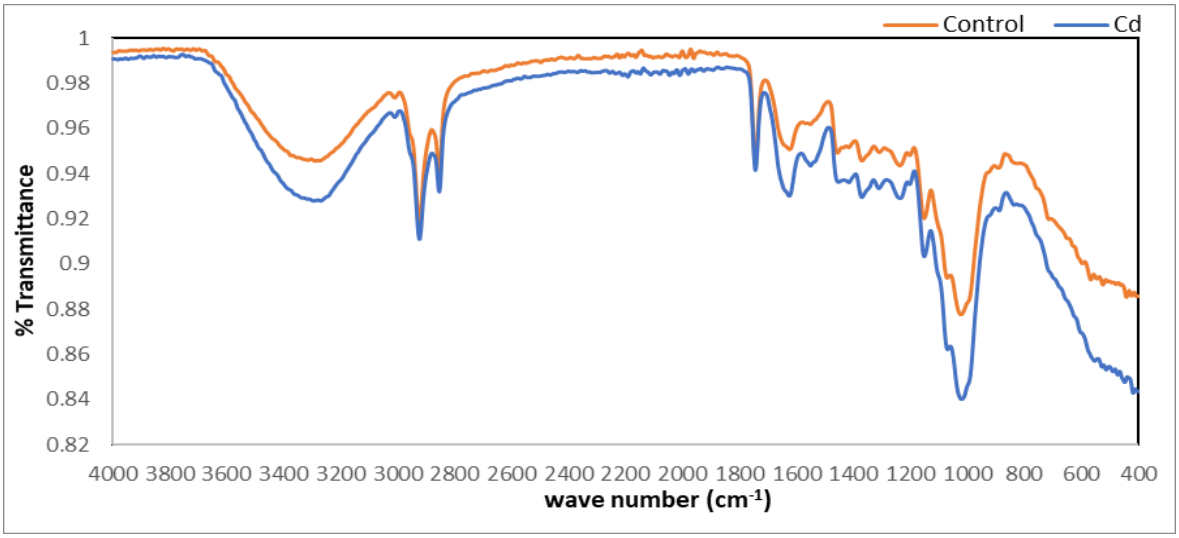

Fig. 6. FTIR spectra of *Alternaria tenuissima* biomass before Cd biosorption (control) represented in orange color and after Cd biosorption (Cd- treated) represented in blue color

5. Effect of fungal treatment on the general health and body weight of male albino rats

Throughout the experiments, no mortality occurred in any of the studied groups. The group exposed to Cd-polluted waters was less active than the negative control rats and had decreased appetite due to less water and food consumption resulting in overall body weakness. Consequently, the net body weight was significantly reduced ($P< 0.001$) in this group (Group B; before water treatment) (Table 3). The adverse effects of cadmium were hypothesized and correlated with the results of **Kim *et al.* (2018)** and **Domouky (2022)**. According to **Eriyamremu *et al.* (2005)**, the reduction in body weight in Cd-polluted water group is likely the result of nutrient loss, significant malabsorption, and a possible decrease in food consumption. Cadmium and other heavy metals have harmful effects on gut flora, which has serious consequences for both food digestion and host health **(Monachese *et al.*, 2012)**. Group C rats given the fungal treatment behaved normally and consumed the same quantity of food and water as their corresponding rats in Group A. The weight gain in the rats in the group receiving water after fungus treatment was considerably significantly higher than that of the rats in the Cd-polluted water group before treatment ($P< 0.01$). These results suggest that cadmium biosorption by fungi was the likely cause of the observed increase in body weight.

Table 3. Effect of fungal treatment on body weight in male albino rats

Treatment	Initial body weight (g)	Final body weight (g)	Gain in body weight (%)	The gain to normal control (%)
Group (A)	115± 2.23	177 ± 6.44	54.1 ± 6.1	100
Group (B)	133 ± 2	130.6 ± 2.20	-1.79 ± 1.13†	-3.30
Group (C)	110 ± 3.16	142 ± 3.39	29.46 *	54.45

The mean ± SE is used to express values. Significance indicated by † for the negative normal control group, where, †$P< 0.05$. Significance indicated by * for the Cd-polluted water group, where, *$P< 0.05$.

6. Effect of fungal treatment on organ coefficient in male albino rats

Table 3 displays the relative organ weights of the liver, spleen, and brain in the Cd-polluted water group (Group B), indicating a significant increase compared to the control group (Group A). Heavy metal buildup in the affected organs has been implicated in these organs **(Ibrahim *et al.*, 2012)**. Some organs (heart, lung, epididymis, seminal vesical, and prostate) showed non-significant changes in the Cd-polluted water group (Group B) compared to the normal control group (Group A) (Table 4). Rats absorb heavy metal (Cd) orally, which could explain these outcomes **(Iqbal *et al.*, 2021)**. In comparison to the control group, the testicular index was significantly lower in the Cd-polluted water group (Group B). According to studies by **El-Demerdash *et al.* (2004)** and **Acharya *et al.* (2008)**, the reproductive organs may atrophy due to oxidative damage and lipid peroxidation caused by Cd. This is because cadmium can cause the testicular tissue to become necrotic

and degenerative, resulting in a decrease in testicular weight (**El-Shahat *et al*., 2009; De Souza Predes *et al*., 2010)**.

Table 4. Effect of fungal treatment on organ coefficient in male albino rats

Groups	Organ coefficient (%)									
	Liver	**Kidney**	**Heart**	**Lung**	**Spleen**	**Brain**	**Testis**	**Epididymis**	**Seminal vesicula**	**Prostate glands**
Group (A)	3.96±0.18	0.44 ± 0.02	0.377±0.03	0.58±0.02	0.58±0.12	0.69±0.019	0.63±0.03	0.186±0.02	0.17±0.02	0.098±0.007
Group (B)	5.2±0.12†	0.38±0.027	0.412±0.013	0.86±0.09	0.94±0.18†	0.98±0.060††	0.4±0.06†	0.2±0.02	0.11±0.027	0.09±0.02
Group (C)	3.7±0.18*	0.38±0.014	0.403±0.09	0.802±0.103	0.64±0.11	0.937±0.038	0.66±0.07*	0.187±0.03	0.113±0.007	0.066±0.009

The mean ± SE is used to express values. Significance indicated by † for the negative normal control group, where, † $P< 0.001$. Significance indicated by * for the Cd-polluted water group, where, *: $P< 0.001$.

7. Effect of fungal treatment on liver and kidney functions of male albino rats

The effect of toxicity on the liver and kidney can be assessed by using biomarkers since changes in these organs impact numerous other bodily processes **(Smaoui *et al*., 2000)**. Hepatic dysfunction and hepatotoxic damage are typically diagnozed by measuring AST and ALT activity, total protein, and albumin levels **(Taha, 2022)**. In the Cd-polluted water (Group B), ALT and AST activities were considerably higher compared to the normal control group (Group A), while total protein and albumin levels exhibited a significant reduction (Fig.7 a, b, c, d) indicating hepatotoxicity. Similar conclusions were grasped by **Fan *et al*. (2018)** and **Baş *et al*. (2021)**, suggesting that these fluctuations may be due to Cd-induced liver damage. There was a correlation between the changes in albumin levels and shifts in the plasma's total protein content. Inhibition of protein synthesis by specific enzymes in cellular processes and the limited substantial excretion of hormones that regulate protein biosynthesis may contribute to the decline in plasma total soluble protein and albumin levels **(Murray *et al*., 2006)**. Renal tissue eliminates metabolic waste products, including creatinine, uric acid, and urea. Certain biochemical markers can be used to diagnose damage caused by heavy metals (**Baş *et al*., 2021)**. Urea, uric acid, and creatinine levels were significantly elevated ($P< 0.001$) in Group B, which received Cd-polluted water orally, compared to Group A, the normal control group, indicating kidney injury (Fig. 7e, f, g). This finding aligns with previous research by **Baş *et al.* (2021)**, providing conclusive evidence of the damaging effects of Cd on the kidney and liver tissues. Compared to the Cd-polluted water (Group B) before water treatment, rats receiving the fungal biosorption treatment (Group C) exhibited significantly lower values of serum AST and ALT activity, creatinine, urea, and uric acid, while total protein and albumin levels were significantly higher. These results indicate that fungal treatment restores liver and kidney functions to a normal range and that fungal treatment improves their overall functionality.

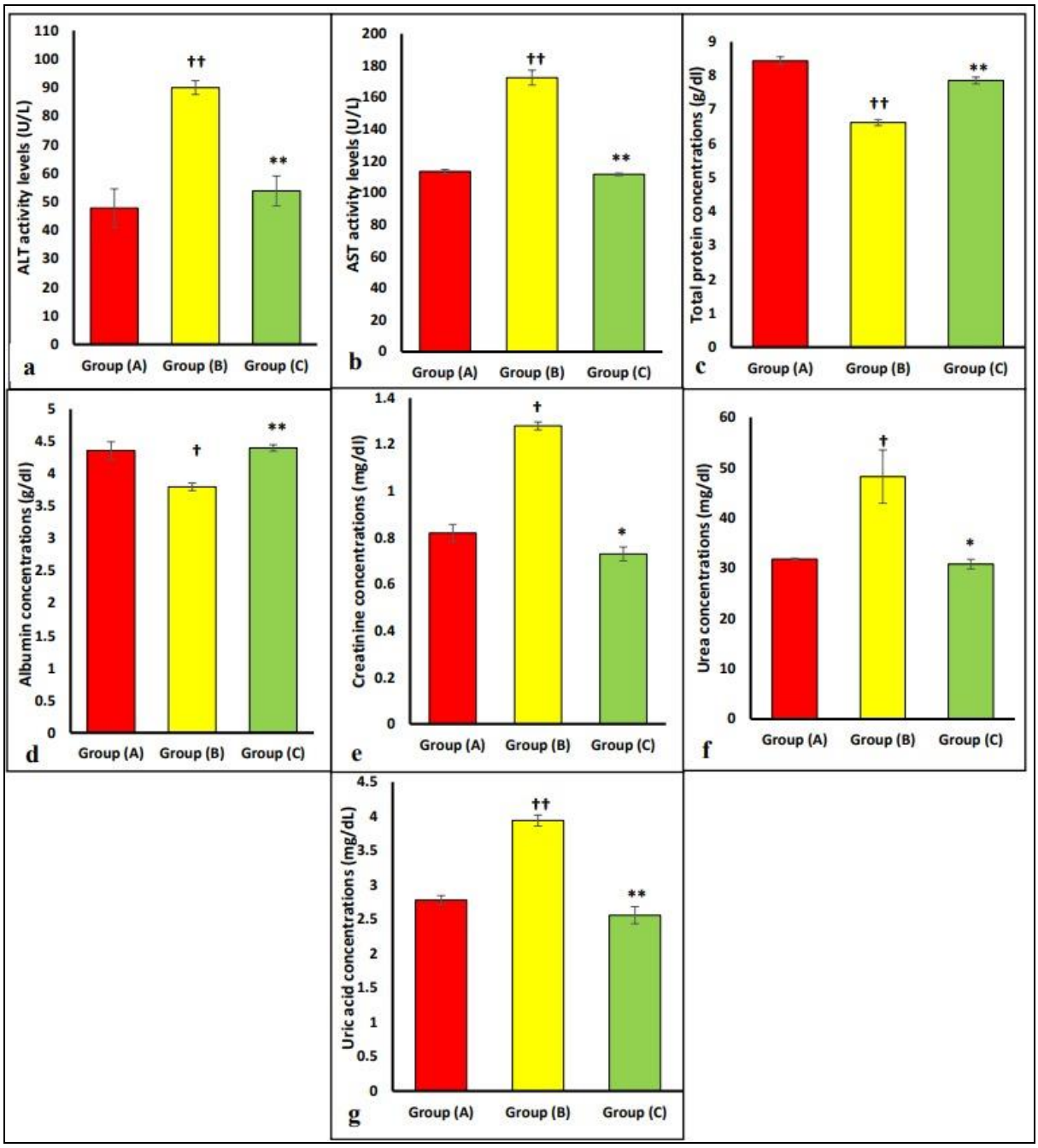

Fig.7. Effect of fungal treatment on liver and kidney function in male albino rats showing: a) ALT activity level, b) AST activity level, c) Total protein concentrations, d) Albumin concentrations, e) Creatinine concentrations, f) Urea concentrations, and g) Uric acid concentrations. ALT stands for alanine aminotransferase, while AST stands for aspartate aminotransferase. The mean ± SE is used to express values. Significance is indicated by † for the negative normal control group, where † $P< 0.05$ and †† $P< 0.001$. Significance is indicated by *indicates significance for the Cd- polluted water group, where,* $P< 0.05$ and **: $P< 0.001$

8. Effect of fungal treatment on cadmium bioaccumulation in the liver and kidney tissues in male albino rats

Since detoxification and excretion are functions of the liver and kidneys, respectively, we examined the effects of cadmium on these organs. In the current investigation, cadmium contamination was found in the normal control group (Group A), but only in the liver; it was undetectable in the kidneys (Table 5). This agrees with the findings of **Ezedom *et al.* (2020)**. These results lead to the hypothesis that the water and/ or diet used was infected with Cd since the control rats were found to have cadmium in their livers despite being supposedly metal-free. The widespread presence of cadmium in today's environment may be to blame **(Tchounwou *et al*., 2012)**. In rats of the Cd-polluted water group, cadmium concentrations in liver and renal tissue were found to be greater in Group B, compared to the control group (Group A) (Table 5). These findings suggest that the high levels of free thiol groups in these proteins were responsible for their potent ability to bind metal ions **(Razavian & Rabiee, 2014)**. After being orally administered for a month, cadmium was shown to accumulate more strongly in the liver than in the kidneys in this investigation, similar to what was found by **Ezedom *et al.* (2020)**. These findings agree with prior experimental investigations showing that metals accumulated in the liver rather than the kidneys after oral and subcutaneous treatment **(Pari & Murugavel, 2005; Asagba, 2010)**. Furthermore, these metals are gradually mobilized from the liver to the kidney as exposure time increases **(Ercal *et al*., 2001; Smith *et al*., 2001)**. Another possible reason is that the cadmium forms a stable combination with the thionein protein in the liver, now known as metallothionein (MT), which plays a significant role in the continued metabolism of this metal after absorption from the digestive system. The kidney is responsible for excreting this metallothionein **(Ruttkay-Nedecky *et al*., 2013; Yang & Shu, 2015)**. Liver and kidney tissues from the group exposed to fungal treatment (Group C) showed no detectable levels of cadmium (Table 5). This indicates that cadmium was removed by using fungal biosorption treatment in the water.

Table 5. Effect of fungal treatment on bioaccumulation of heavy metals in the liver and kidney of male albino rats

Treatments	**Cadmium concentrations (mg/ kg)**	
Selected tissues	**Liver**	**Kidney**
Group (A)	0.2±0.12	ND
Group (B)	1.09±0.026†	0.83±0.081†
Group (C)	ND	ND

The mean ± SE is used to express values. † indicates significance with respect to the negative normal control group. Where, † $P < 0.001$. ND: Not detectable.

9. Effect of fungal treatment on semen parameters in male albino rats

Group B, which was exposed to Cd-polluted water, had significantly lower sperm counts and motility and significantly more sperm abnormalities compared to Group A, which was exposed to double distilled water (Table 6). These findings align with the previous study of **De Franciscis *et al.* (2015)**. Monitoring sperm count and motility are crucial criteria for determining an animal's fertility (**Taha & Soliman, 2019; Taha & Gouda, 2022)**. The number of sperm abnormalities was significantly higher in the Cd-polluted water group (Group B) than that recorded in the normal control group (Group A) and significantly lower in the fungal biosorption water treatment group (Table 7).

The excessive generation of oxidative stress byproducts leading to cellular death may be responsible for the significant increase in sperm abnormalities **(Sen-Gupta *et al.*, 2004; El-Shahat *et al.*, 2009)**. After fungal treatment (Group C), sperm count and motility returned to normal compared to the corresponding Cd-polluted water group (Group B), and the increase was statistically significant, and there was also a significant decrease in sperm abnormalities (Tables 6, 7). These findings suggest that fungal biosorption is an effective method for removing heavy metals from water.

Table 6. Effect of fungal treatment on semen count and motility in male albino rats

Treatments	Sperm count × 10^6mL	Sperm motility (%)		Immotile sperm
		Progressive motile sperm	Non-progressive motile sperm	
Group (A)	158± 4.8	20.6± 1.86	53.2± 4.16	26.2± 3.6
Group (B)	32.2± 4.9†	7.2± 1.11†	21.8± 4.06†	71± 4.01†
Group (C)	71.4± 8.2*	7.2± 0.5	53.8± 3.4**	39± 3.30**

The mean ± SE is used to express values. Significance indicated by † for the negative normal control group, where, † $P< 0.05$; and ††$P< 0.01$; ††† $P< 0.001$. Significance indicated by * for the Cd-polluted water group, where, $P< 0.05$; and **: $P< 0.001$.

Table 7. Effect of fungal treatment on semen morphology in male albino rats

Treatments	Normal sperm (%)	Sperm abnormalities (%)					
		Head abnormalities sperm (%)		Tail abnormalities sperm (%)			Total sperm abnormalities (%)
		Amorphous	Headless	Coiled	Highly coiled	Tailless	
Group (A)	79.6± 2.4	3.6± 0.87	6.2± 0.7	3.6± 1.2	3.6± 0.4	3.6± 0.8	20.4± 2.4
Group (B)	68.8± 1.1†††	5.8± 0.3	7.6± 0.5	7.6± 0.67††	4.6± 0.9	5.6± 0.9†	31.2± 1.1†††
Group (C)	82.6± 0.5***	5.4± 1.2	4.8± 0.8*	3± 0.89*	1± 0.3**	2.8± 1.06	17.4± 0.50**

The mean ± SE is used to express values. Significance indicated by † for the negative normal control group, where, † $P< 0.05$; and ††$P< 0.01$; ††† $P< 0.001$. Significance indicated by * for the Cd-polluted water group, where, $P< 0.05$; and **: $P< 0.001$.

10. Effect of fungal treatment on gross and histopathological evaluations

10.1. Liver

In the healthy control group, the livers appeared to be typical in size and color on a gross morphological level. Liver sections from this group showed normal histological structure, without any obvious abnormalities when examined under the microscope (Fig. 8a).

The livers of the Cd-polluted water group (Group B) were smaller and paler in gross morphology compared to the untreated group. Histopathological examination of this group revealed the presence of portal mononuclear inflammatory cells along with limited portal fibrosis characterized by the extension of fibrous strands from the portal triads, and hepatic necrosis was determined in hepatic parenchyma (Fig. 8b-f). Hepatopthological results are in accordance with other earlier researches **(Ibrahim *et al*., 2014; Saravpreet *et al*., 2018)**. These findings may be traced back to a decline in membrane permeability caused by the development of a hepatic lesion **(Layachi & Kechrid, 2012; Diaby *et al*., 2016)**. In cadmium toxicity, it is also said to be a cellular lesion brought on by cell necrosis **(Wakeel *et al*., 2020)**. Since cadmium primarily localizes in the hepatocytes, its accumulation there may lead to cell death in the liver and lymphocytic infiltration **(Tawari-Fufeyin *et al*., 2008)**.

There was a marked improvement in the gross morphologies of the livers of rats in the fungal treatment group (Group B), which appeared normal in size and color. Histopathological examination of this group revealed hepatic tissue with normal hepatocytes arranged in hepatic cords, intact hepatic sinusoids, and diffuse mild vacuolation in hepatocytes within the hepatic parenchyma with pale cytoplasm (Fig. 8g-h). This suggests that the liver structure recovered to its normal state after the water treatment. However, in the Cd group, examination of hepatic parenchyma revealed diffuse mild vacuolation hepatocytes with pale cytoplasm. Protein synthesis inhibition, ATP depletion, and microtubule disaggregation/ changes in substrate use have all been linked to hepatocyte vacuolation **(Hinton & Lauren, 1990)**.

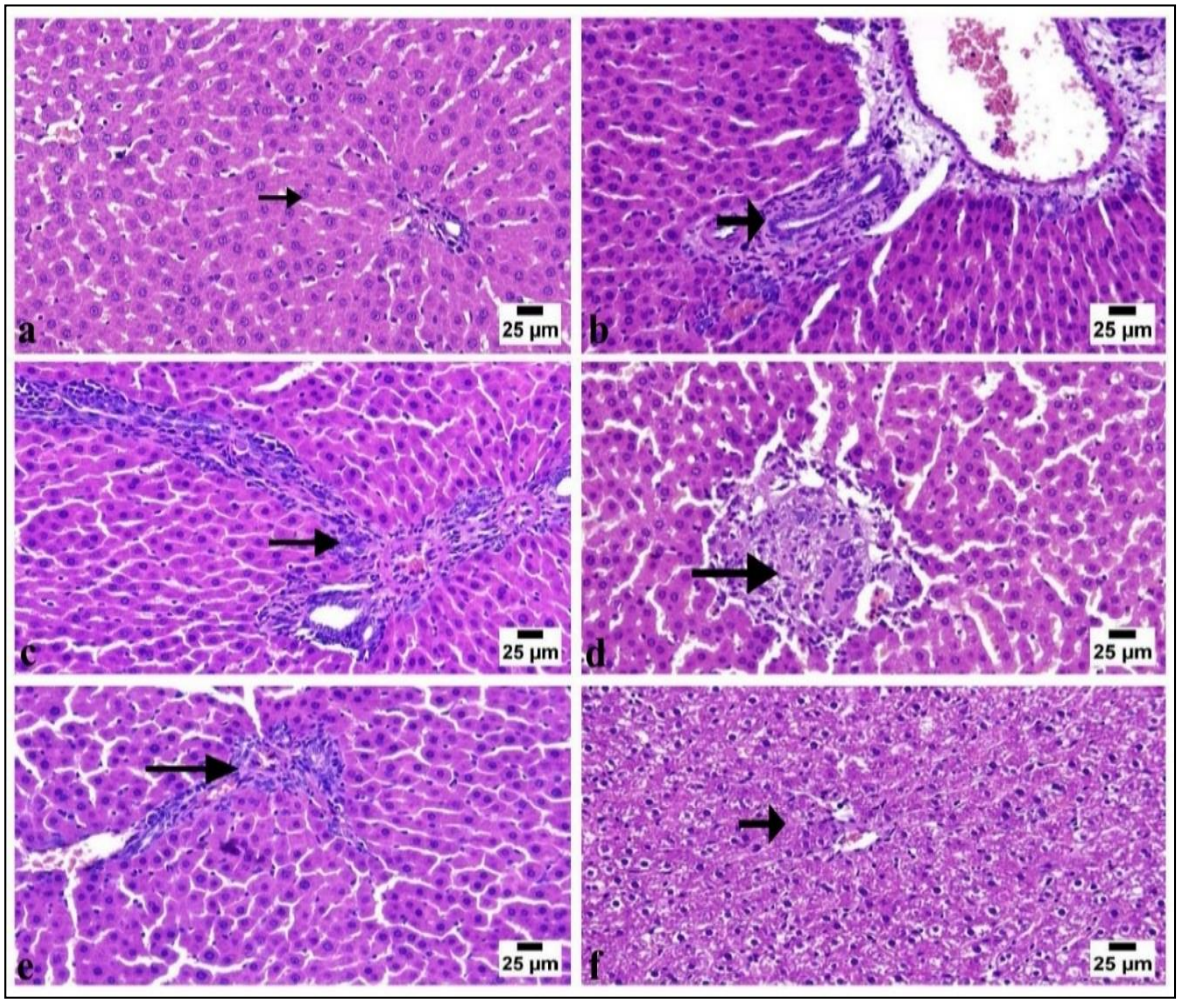

Fig. 8. Photomicrographs of liver tissue sections from albino rats stained with (H & E) showing: (a) The negative control group exhibits the typical histological organization of hepatic parenchyma (arrow). (b-f) In the Cd-polluted water group, (b) Portal fibroplasia with proliferation of biliary epithelium (arrow) is observed, (c) Fibrous strands extending from portal triads (arrow) are evident, (d) Focal hepatic necrosis with infiltration of inflammatory cells (arrow) is visible, (e) Limited portal fibroplasia with infiltration of inflammatory cells (arrow) is seen. (f) In the fungal treatment group, vacuolation of hepatic parenchyma (arrows) is evident

10.2. Kidney

Similar to the control group, the gross morphology of the kidneys in all treatment groups was clearly normal in terms of size and color.

Both the renal cortex and medulla seemed normal under the microscope when examining kidney sections from the control group (Fig. 9a, b). The kidney sections of Cd-polluted water rats (Group B) revealed marked disruption of the tissue architecture along with extensive multifocal perivascular edema and inflammatory cell infiltration associated with multifocal areas of interstitial nephritis in both renal cortex and renal medulla.

Cystically dilated renal tubules lined with attenuated epithelial cells were detected in the corticomedullary junction and renal cortex. In addition, accumulation of renal casts were detected in the tubular lumen in the Cd-polluted water group (Fig. 9c- g). These results are consistent with the findings of **Ibrahim *et al.* (2014)**. The tubular epithelium suffered from vacuolar degeneration. These results coincide with those of **Prenika *et al*. (2021)**. It has been postulated that kidneys are the target organs for harmful consequences of the cadmium **(Wang *et al*., 2014; Satarug, 2018)**, as it accumulates, leading to kidney damage **(Poontawee *et al*., 2016)**. In addition, the toxicity of cadmium causes tubular dysfunction leading to reduced glomerular filtration, which results in kidney failure **(Fahim *et al*., 2012)**. Marked improvement was determined in a macroscopic examination of the kidney of the fungal treatment group (Group C), which showed normal renal cortex and medulla (Figs. 9h-i). This suggests that the kidney structure recovered to its normal structure after fungal biosorption water treatment.

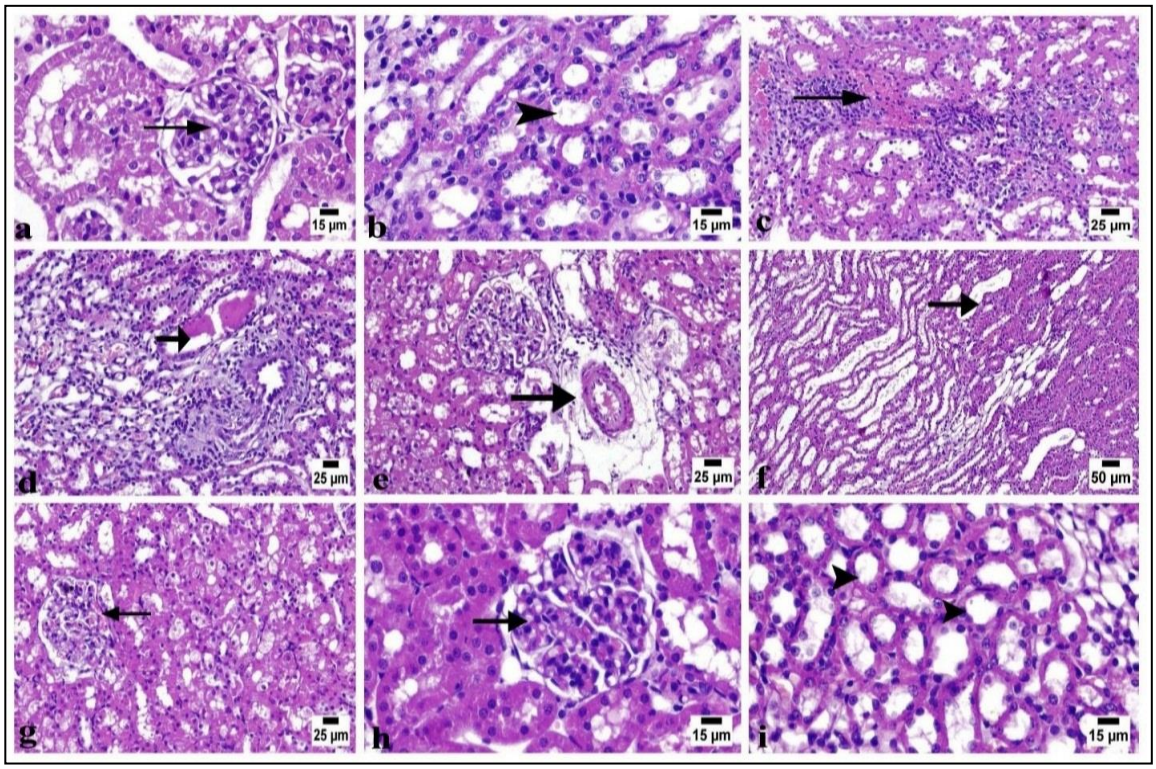

Fig. 9. Photomicrographs of sections of kidney tissue from albino rats stained with (H & E) showing: (a & b) The negative control group showed a normal renal cortex (arrow) and normal renal medulla (arrowhead). (c-g) Cd-polluted water group showed (c) Multifocal interstitial nephritis in the renal cortex (arrow), (d) Focal interstitial nephritis in the renal medulla associated with an accumulation of renal cast in the tubular lumen (arrow), (e) Perivascular edema and infiltration of inflammatory cells (arrow), (f) Cystic dilation of renal tubules (arrow), (g) Vacuolar degeneration of the epithelium lining renal tubules in the renal cortex (arrow), (h-i) The fungal treatment group showed a normal renal cortex (arrow) and a normal renal medulla (arrowhead)

10.3. Testis

Contrasted with the control rats, the testis morphology of Cd-polluted water rats exhibited a smaller size and a paler color. Testicular examination of testis from this group revealed the usual histological structure of seminiferous tubules, which appeared to be packed with increasing numbers of spermatogonial cells and Sertoli cells of many spermatids and sperm present (Fig. 10a, b).

A testicular examination of the Cd-polluted water rats (Group B) revealed excessive exfoliation of the germinal epithelium and severe testicular degeneration. There was also a significant reduction in the diameter and epithelial height of seminiferous tubules (atrophied tubules), as well as a significant decrease in John's score compared to the normal control testis. Additionally, there was congestion of interstitial blood vessels (Figs. 9, 10c-g). These findings align with previous reports by **Liu *et al*. (2016)** and **Chen *et al*. (2018).** Moreover, they are consistent with those shown in other studies showing cadmium-induced testicular necrosis **(Thompson & Bannigan, 2008; De Souza Predes *et al*., 2010)**. Cadmium disrupts cell-to-cell endothelial and epithelial connections and causes blood leakage into the testis **(Cheng *et al*., 2011; Lafuente, 2013)**. The testis is more susceptible to Cd than other vital organs, as demonstrated by numerous investigations into the implications of Cd pollution. Even low doses that are harmless to the body as a whole can disrupt the testicular function **(Blanco *et al*., 2007)**. Metallothionein (MT), a protein involved in cadmium poisoning, may be responsible for this. MT is a member of the cysteine- rich metal-binding protein family and acts as a Cd detoxifier. When there is insufficient MT to bind with Cd, oxidative stress and disruption of spermatogenesis can occur, leading to toxic consequences **(Xu *et al*., 2005)**. Cadmium-induced testicular and cellular damage negatively affects by damaging the testicular germinal epithelium, Leydig cells, and Sertoli cells **(Elbaghdady *et al*. 2018)**. Exposure to Cd also alters germ cell adhesion and disrupts the tight junctions between Sertoli cells, causing immature cells to be shed into the lumen of the seminiferous tubules. This resulted in a reduction in the number of viable sperm and the increase in epithelial and endothelial permeability (**Wong & Cheng, 2011**). Damage to Sertoli cells and disrupted cell connections are evident in the shedding of germ cells into the tubular lumen **(Akhtar *et al*., 2009)**. Regarding the shedding of the cells into the lumen, there is a decrease in lumen size, and the epithelial volume fraction decreases significantly. Vacuole development in Sertoli cells and a near- total absence of spermatozoa suggest impairment of spermatogenesis **(Creasy, 2001)**. Cd may suppress spermatogenic cells, leading to a reduction in the number of spermatogenic cells in the seminiferous tubules **(Yari *et al*., 2010)**. Previous research has documented the beneficial effects of antioxidants on Cd-induced testicular damage in animals **(Kara *et al*., 2007; Yadav & Khandelwal, 2008)**. The gross morphology of the testis in the treated Group C has significantly improved, returning to normal in size and color. Macroscopic examination revealed normal testicular

tissue with a considerable increase in diameter, the epithelial height of seminiferous tubules, and John's score (Fig. 10h-i, 11).

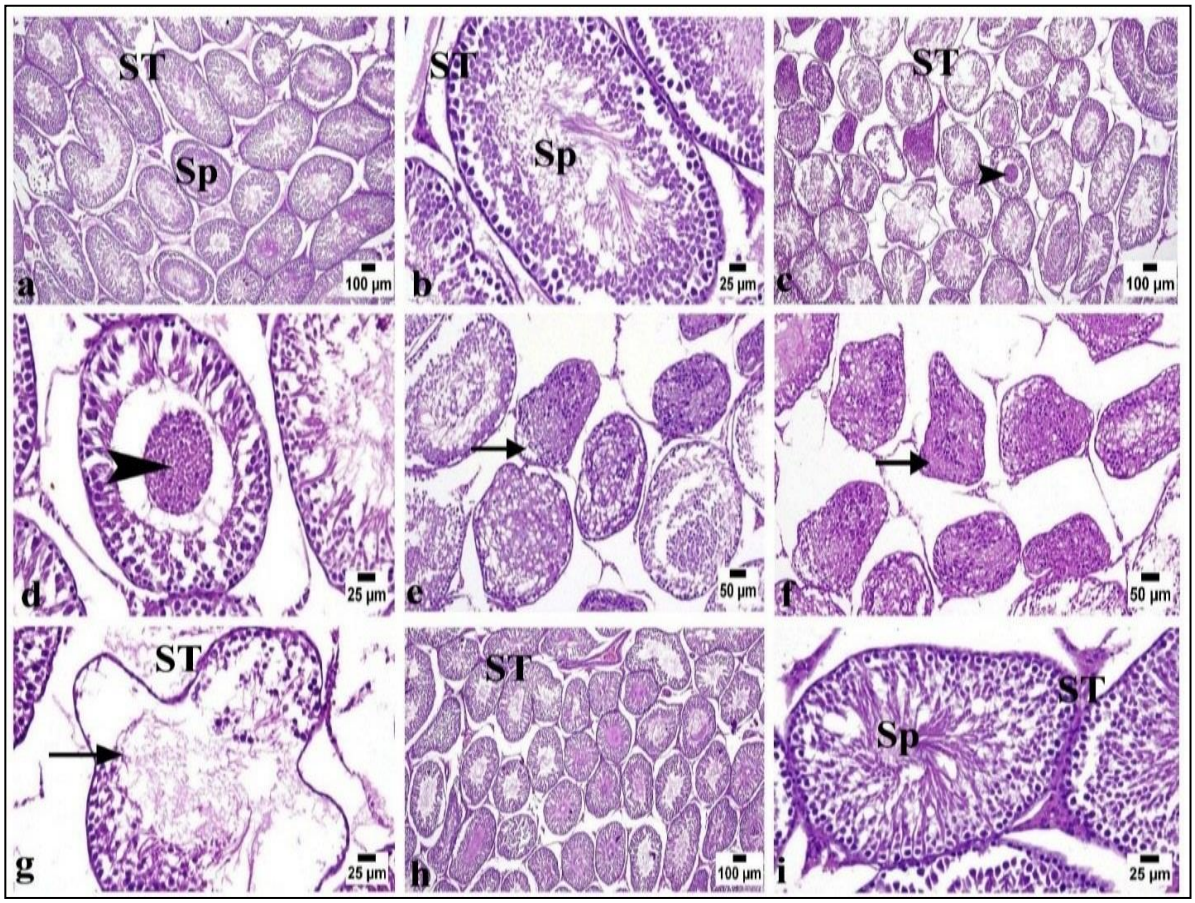

Fig. 10. Photomicrographs of sections of testis tissue from albino rats stained with H & E showing: (a & b) The control group showed a normal testicular structure consisting of stratified germinal epithelium consisting of many ovals or rounded seminiferous tubules (ST). These tubules displayed all stages of spermatogenesis including primary spermatocytes, spermatids, and a lumen filled with abundant spermatozoa (Sp), (c-g) Cd-polluted water group displayed (c & d) seminiferous tubules (ST) with excessive exfoliation of germinal epithelium (arrowhead), (e-g) Furthermore, the Cd-polluted water group showed severe testicular degeneration (arrow), (h-i) In the fungal treatment group, normal seminiferous tubules (ST) containing numerous sperms (Sp) were observed.

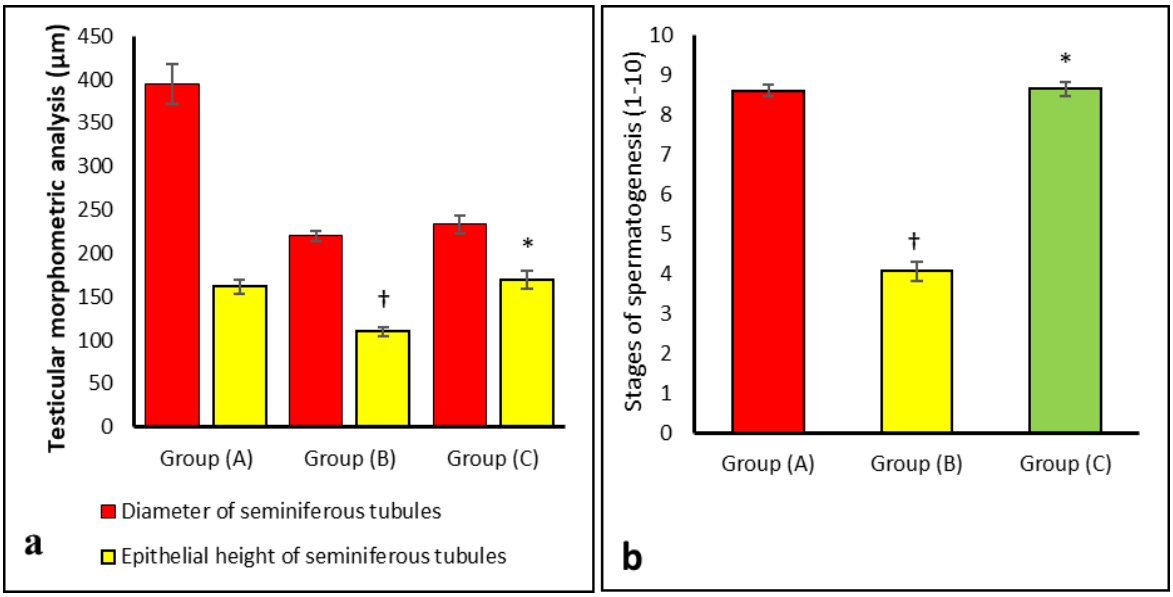

Fig.11. Effect of fungal treatment on a) Morphometric analysis of testis in male albino rats and b) Stages of spermatogenesis. The mean ± SEM is used to express values. Significance indicated by † for the negative normal control group, where, † $P< 0.001$. Significance indicated by * for Cd positive control group, where *: $P< 0.001$

10.4. Epididymis

Noticeable gross morphology of epididymis is the same in all treatment groups, as compared to normal control ones. Epididymal microscopic examination of the control rats showed the normal histological structure of epididymis, and epididymal ducts containing increased amounts of formed spermatozoa (Fig. 12).

Histopathological examination of epididymis from the Cd-polluted water group showed vacuolation of epididymal epithelium with marked atrophied duct and hypospermia, and the presence of sperm stasis in ducts (Fig. 12b-g). Marked improvement in the macroscopic examination of the epididymis of rats, injected orally with water after fungal biosorption treatment, showed normal epididymal ducts with numerous spermatozoa and intact epididymis without any obvious abnormalities (Fig. 12h-i).

Histopathological results of the epididymis tissue of Cd-polluted water group rats showed reduced sperm content accompanied by a significant reduction in the ductal lumen size (ductal atrophy) due to the reduced volume of sperm and fluid (Fig. 12b-g, 13). Furthermore, the epididymal epithelium in the Cd-group is vacuolated, and there is substantial atrophic duct hypospermia and sperm stasis. Cd-intoxication increases epididymal lipid peroxidation damage and leads to sperm and reproductive system-wide variations in antioxidant levels, which these findings relate to oxidative stress and lipid peroxidation **(Paydın *et al.*, 2021)**. A marked improvement in the epididymis of rats injected orally with Cd-contaminated water after fungal biosorption treatment showed an ameliorative effect on numerous numbers of mature spermatozoa in epididymal ducts, with a significant increase in the diameter of epididymal ducts (Figs. 12h- i, 13).

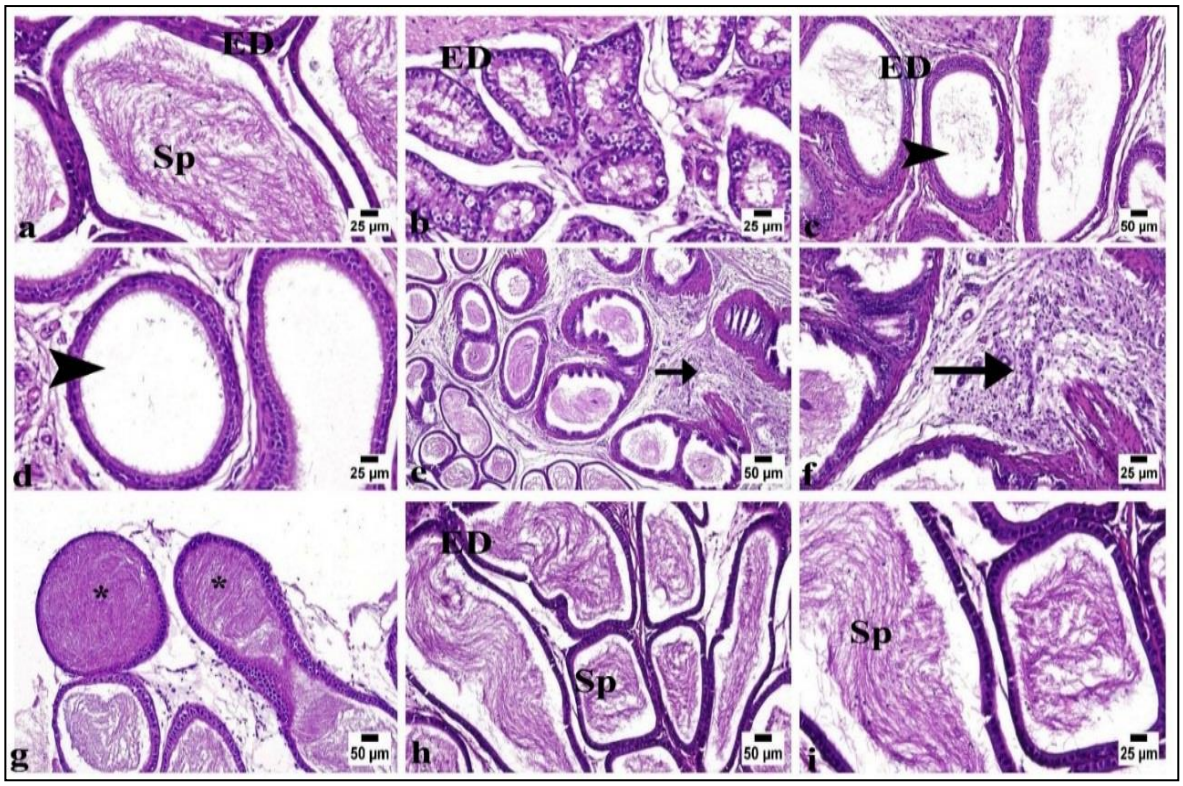

Fig. 12. Photomicrographs of sections of epididymis tissue from albino rats stained with (H & E) showing: (a) The control group shows epididymal tubules (ED) filled with spermatozoa in the ductal lumen (Sp), (b-g) The Cd-polluted water group exhibits atrophied epididymal ductal (ED) with hypospermia (arrowhead) and presence of interstitial epididymitis (arrow), the presence of sperm stasis (*), and congested blood vessel (BV), and (h-i) The fungal treatment group displays epididymis with epididymal containing mature spermatozoa

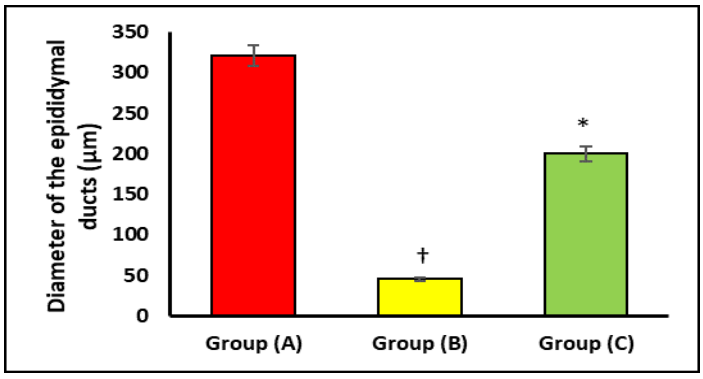

Fig.13. Effect of fungal treatment on diameter of epididymal ducts of male albino rats. The mean ± SE is used to express values. Significance indicated by † for the normal control group, where † $P< 0.001$. Significance indicated by * for the Cd-polluted water group, where, * $P< 0.001$

CONCLUSION

Biosorption of heavy metals from the environment is an effective, affordable, and environmentally sound option. Regarding its unique metal binding properties, fungal dead biomass may provide a low-cost method for removing dangerous heavy metals from contaminated waters. Our research demonstrated that under optimal conditions, the dead biomass of the cadmium-resistant fungus *Alternaria tenuissima* could biosorb cadmium ions achieving an efficiency up to 99.95%. Furthermore, the treated aqueous solution did not alter any biochemical parameters, causing Cd accumulation in the liver and kidney, or inducing any change in the histopathology of organs, such as the liver, kidney, testis, and epididymis. These results indicate that *A. tenuissima* holds promise as biological option for on-site Cd remediation, as it can improve environmental conditions without affecting animals. In future research, it is recommended to explore the use of immobilized dead biomass of *A. tenuissima* for adsorbing Cd from polluted water.

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Conflict of interest

None declared

Author Contribution Statement

Gouda SA and Taha A: Conceived and designed the experiments; Gouda SA, Taha A, Eid DM, Elsharkawy TMF, Mohamed FS, Mostafa SI, and Hussein SA: Performed the experiments; Gouda SA and Taha A: Analyzed, interpreted the data, contributed materials, and wrote the paper equally. All authors have read and agreed to the published version of the manuscript.

REFERENCES

Acharya, U.R.; Mishra, M.; Patro, J. and Panda, M.K. (2008). Effect of vitamins C and E on spermatogenesis in mice exposed to cadmium. Reprod. Toxicol. (Elmsford, N.Y.), 25(1): 84–88. DOI: 10.1016/j.reprotox.2007.10.004.

Akhtar, N.; Srivastava, M.K. and Raizada, R.B. (2009). Assessment of chlorpyrifos toxicity on certain organs in rat, *Rattus norvegicus*. J. Environ. Biol., 30(6): 1047–1053.

Akhtar, S.; Mahmood-ul-Hassan, M.; Ahmad, R.; Suthor, V. and Yasin, M. (2013). Metal tolerance potential of filamentous fungi isolated from soils irrigated with untreated municipal effluent. Soil Environ., 32: 55–62.

Ali, E.A.M.; Sayed, M.A.; Abdel-Rahman, T.M.A. and Hussein, R. (2021). Fungal remediation of Cd (II) from wastewater using immobilization techniques. RSC Advances, 11(8): 4853–4863. DOI: 10.1039/D0RA08578B.

Alzahrani, N.H.;Alamoudi, K.H. and El Gendy, M. M.A.A. (2017). Molecular Identification and Nickel Biosorption with the Dead Biomass of Some Metal Tolerant Fungi. J. Microb. Biochem. Technol., 9: 6. DOI: 10.4172/1948-5948.1000383.

American Public Health Association (APHA-3111B) (2017). Standard methods for the examination of water and wastewater. edition. Rice, E.W.; Baird, R.B. and Eaton, A.D (23rd Eds.). Washington, D.C., pp. 40.

Andersson, G.B. (1999). Epidemiological features of chronic low-back pain. Lancet (London, England), 354(9178): 581–585. DOI: 10.1016/S0140-6736(99)01312-4.

Aracagök, Y.D.; Öğün, E.; Torun, M.; Akyıl, H.; Cihangir, N. and Sanin, S. (2021). Fungal biosorption of cadmium (II) onto *Fennelia nivea* from aqueous solution: equilibrium, thermodynamics, and kinetics. Desalin. Water Treat. 222: 386–393. DOI: 10.5004/dwt.2021.27091.

Asagba, S.O. (2010). A comparative study on the biochemical effect of ocular and oral cadmium administration in rabbits. Afr. J. Biotechnol., 9(21): 3016–3025.

Bahafid, W.; Joutey, N.T.; Asri, M.; Sayel, H.; Tirry, N.; El Ghachtouli, N. and Sayel, N.T. H. (2017). Yeast biomass: an alternative for bioremediation of heavy metals. Yeast-Industrial Applications, 559: 269–273. DOI: 10.5772/intechopen.70559.

Bahobil, A.; Bayoumi, R.; Atta, H. and El-Sehrawey, M. (2017). Fungal biosorption for cadmium and mercury heavy metal ions isolated from some polluted localities in ksa. Int. J. Curr. Microbiol. Appl Sci., 6(6): 2138–2154. DOI: 10.20546/ijcmas.2017.603.253.

Barros, L.M.; Macedo, G.R.; Duarte, M.M.L.; Silva, E.P. and Lobato, A.K.C.L. (2003). Biosorption of cadmium using the fungus *Aspergillus niger*. Braz. J. Chem. Eng., 20(3): 229–239. DOI: 10.1590/s0104-66322003000300003.

Baş, H.; Apaydın, F. G.; Kalender, S. and Kalender, Y. (2021). Lead nitrate and cadmium chloride induced hepatotoxicity and nephrotoxicity: Protective effects of sesamol on biochemical indices and pathological changes. J. Food Biochem., 45(7): e13769. DOI: 10.1111/jfbc.13769.

Bergmeyer, H.U.; Scheibe, P. and Wahlefeld, A.W. (1978). Optimization of methods for aspartate aminotransferase and alanine aminotransferase. Clin. Chem., 24(1): 58–73.

Blanco, A.; Moyano, R.; Vivo, J.; Flores-Acuña, R.; Molina, A.; Blanco, C.; Agüera, E. and Monterde, J.G. (2007). Quantitative changes in the testicular structure in mice exposed to low doses of cadmium. Environ. Toxicol. Pharmacol., 23(1): 96–101. DOI: 10.1016/j.etap.2006.07.008.

Broerse, M.; Oorsprong, H. and Van Gestel, C.A. (2012). Cadmium affects toxic kinetics of pyrene in the collembolan *Folsomia candida*. Ecotoxicol., 21(3): 795–802.

Chen, N.; Su, P.; Wang, M. and Li, Y.M. (2018). Ascorbic acid inhibits cadmium-induced disruption of the blood-testis barrier by regulating oxidative stress-mediated p38 MAPK pathways. Environ. Sci. Pollut. Res. Int, 25(22): 21713–21720. DOI: 10.1007/s11356-018-2138-4.

Cheng, C.Y.; Wong, E.W.; Lie, P.P.; Li, M.W.; Su, L.; Siu, E.R.; Yan, H.H.; Mannu, J.; Mathur, P.P.; Bonanomi, M.; Silvestrini, B. and Mruk, D.D. (2011). Environmental toxicants and male reproductive function. Spermatogenesis, 1(1): 2–13. DOI:10.4161/spmg.1.1.13971.

Cho, D.H.; Kim, E.Y. and Hung, Y.T. (2010). Heavy metal removal by microbial biosorbents. In: "Handbook of Environmental Engineering". Wang *et al*. (Eds). Springer Science Business Media 11, pp 375–402.

Creasy, D.M. (2001). Pathogenesis of male reproductive toxicity. Toxicol. Pathol., 29(1): 64–76. DOI: 10.1080/019262301301418865.

Cuypers, A.; Plusquin, M.; Remans, T.; Jozefczak, M.; Keunen, E.; Gielen, H.; Opdenakker, K.; Nair, A.R.; Munter, E.; Artois, T.J. and Vangronsveld, J. (2010). Cadmium stress: an oxidative challenge. BioMetals, 23: 927–940.

Das, P.; Mahanty, S.; Ganguli, A.; Das, P. and Chaudhuri, P. (2019). Role of Manglicolous fungi isolated from Indian Sunderban mangrove forest for the treatment of metal containing

solution: Batch and optimization using response surface methodology. Environ. Technol. Innov., 13:166–178. DOI: 10.1016/j.eti.2018.11.006.

De Franciscis, P.; Ianniello, R.; Labriola, D.; Ambrosio, D.; Vagnetti, P.; Mainini, G.; Trotta, C.; Mele, D.; Campitiello, M.R. and Caprio, F. (2015). Environmental pollution due to cadmium: measure of semen quality as a marker of exposure and correlation with reproductive potential. Clin. Exp. Obstet. Gynecol., 42(6): 767–770.

De Souza Predes, F.; Diamante, M.A. and Dolder, H. (2010). Testis response to low doses of cadmium in Wistar rats. Int. J. Exp. Pathol., 91(2): 125–131. DOI:10.1111/j.1365-2613.2009.00692.x.

Diaby, V.; Ali, A.A.; Adunlin, G.; Kohn, C.G. and Montero, A.J. (2016). Parameterization of a disease progression simulation model for sequentially treated metastatic human epidermal growth factor receptor 2 positive breast cancer patients. Curr. Med. Res. Opin., 32(6): 991–996. DOI: 10.1185/03007995.2016.1149056.

Domouky, A.M.; Abdel-kareem, R.H. and El-wafaey, D.I. (2022). Zinc Supplementation Attenuates Cadmium-Induced Jejunal Injury in Adult Male Albino Rats: Histopathological and Biochemical Study. Zagazig Univ. Med. J., 28(2): 364–378. DOI 10.21608/zumj.2022.115392.2451.

Doumas, B.T.; Watson, W.A. and Biggs, H.G. (1971). Albumin standards and the measurement of serum albumin with bromcresol green. Clin. Chim. Acta,, 31(1): 87–96. DOI: 10.1016/0009-8981(71)90365-2.

Dua, T.K.; Dewanjee, S.; Khanra, R.; Bhattacharya, N.; Bhaskar, B.; Zia-UlHaq, M. and De Feo, V. (2015). The effects of two common edible herbs, Ipomoea aquatica and *Enhydra fluctuans*, on cadmium-induced pathophysiology: a focus on oxidative defence and anti-apoptotic mechanism. J. Transl. Med., 28: 13245.

El-Sayed, M.T. and El-Sayed, A.S.A. (2020). Tolerance and mycoremediation of silver ions by *Fusarium solani*. Heliyon, 6(5): e03866. DOI: 10.1016/j.heliyon.2020.e03866.

Elbaghdady, H.A.M.; Alwaili, M.A. and El-Demerdash, R.S. (2018). Regenerative potential of bone marrow mesenchymal stem cells on cadmium chloride-induced hepato-renal injury and testicular dysfunction in sprague dawley rats. Ecotoxicol. Environ. Saf., 30(164): 41–49. DOI: 10.1016/j.ecoenv.2018.07.019.

El-Demerdash, F.M.; Yousef, M.I.; Kedwany, F.S. and Baghdadi, H.H. (2004). Cadmium-induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats: protective role of vitamin E and beta-carotene. Food Chem. Toxicol., 42(10): 1563–1571. DOI: 10.1016/j.fct.2004.05.001.

Ellis, M.B. (1971). Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England, 608 pp.

El-Sayed, M.T. (2014). An investigation on tolerance and biosorption potential of *Aspergillus awamori* ZU JQ 965830.1 to Cd(II). Ann. Microbiol., 65(1): 69–83. DOI: 10.1007/s13213-014-0838-7.

El-Sayed, M.T. and Reda, F.M. (2011). Sorption of Cadmium and Lead Ions by *Cunninghamella elagans* Lender ML2. Egypt. J. Microbiol., (46): 213–231. DOI: 10.21608/EJM.2011.272.

El-Shahat, A.; Gabr, A.; Meki, A. and Mehana, El. (2009). Altered testicular morphology and oxidative stress induced by cadmium in experimental rats and protective effect of simultaneous green tea extract. Int. J. Morphol., 27: 757–764. DOI: 10.4067/S0717-95022009000300020.

Ercal, N.; Gurer-Orhan, H. and Aykin-Burns, N. (2001). Toxic metals and oxidative stress part 1: Mechanisms involved in metal-induced oxidative damage. Curr. Top. Med. Chem., 1(6): 529–539. DOI: 10.2174/1568026013394831.

Eriyamremu, G.E.; Asagba S.O.; Onyeneke E.C. and Adaikpoh M.A. (2005). Changes in carboxypeptidase A, dipeptidase and Na+/K+ ATPase activities in the intestine of rats orally exposed to different doses of cadmium. BioMetals, 18(1):1–6.

Ezedom, T.; Asagba, S. and Tonukari, N.J. (2020). Toxicological effects of the concurrent administration of cadmium and arsenic through the food chain on the liver and kidney of rats. J. Basic Appl. Zool., 81(16). DOI: 10.1186/s41936-020-00146-2.

Fahim, M.A.; Nemmar, A.; Dhanasekaran, S.; Singh, S.; Shafiullah, Y.M.; Zia, S. and Hasan, M.Y. (2012). Acute cadmium exposure causes systemic and thromboembolic events in mice. Physiol. Res., 61(1): 73–80. DOI: 10.33549/physiolres.932238.

Fan, R.; Hu, P.; Wang, Y.; Lin, H.; Su, K.; Feng, X.; Wei, L. and Yang, F. (2018). Betulinic acid protects mice from cadmium chloride-induced toxicity by inhibiting cadmium-induced apoptosis in kidney and liver. Toxicol. Lett., 299: 56–66. DOI: 10.1016/j.toxlet.2018.09.003.

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. J. Hazard. Mater., 160 (2-3): 655–661. DOI: 10.1016/j.jhazmat.2008.03.038.

Fazli, M.M.; Soleimani, N.; Mehrasbi, M.; Darabian, S.; Mohammadi, J. and Ramazani, A. (2015). Highly cadmium tolerant fungi: their tolerance and removal potential. J. Environ. Health Sci. Eng., 13(1): 19. DOI: 10.1186/s40201-015-0176-

Felsentein, J. (1985). Phylogenies and the Comparative Method. Journal Storage, 125(1): 1–15. DOI: 10.1086/284325.

Fu, F. and Wang, Q. (2011). Removal of heavy metal ions from Wastewaters: a review. J. Enviro. Manag., 92(3): 407–418. DOI:10.1016/j.jenvman.2010.11.011

Ge, W.; Zamri, D.; Mineyama, H. and Valix, M. (2011). Bioaccumulation of heavy metals on adapted *Aspergillus foetidus*. Adsorption, 17(5): 901–910. DOI: 10.1007/s10450-011-9359-x.

Genchi, G.; Sinicropi, M. S.; Lauria, G.; Carocci, A. and Catalano, A. (2020). The Effects of Cadmium Toxicity. Int. J. Environ. Res. Public Health, 17(11): 3782. DOI: 10.3390/ijerph17113782.

Gouda, S.A. and Taha, A. (2023). Biosorption of Heavy Metals as a New Alternative Method for Wastewater Treatment: A Review. Egypt. J. Aquat. Biol. Fish., 27(2): 135–153. DOI:10.21608/ejabf.2023.284415.

Hassan, S.H.A.; Koutb, M.; Nafady, N.A. and Hassan, E.A. (2018). Potentiality of *Neopestalotiopsis clavispora* in biosorption of cadmium and zinc, Chemosphere, 202: 750–756. DOI: 10.1016/j.chemosphere.2018.03.114.

Hassan, E.A.; Nafady, N.A.; Hassan, S.H.A.; Moustafa, Y.S.; Alamri, S.A. and Hashem, M. (2021). Cadmium biosorption potential and kinetic behavior of endophytic *Fusarium verticillioides* and its green synthesized silver nanoparticles (GSNPs). Globa. Nest. J., 23(3): 449–457. DOI: 10.30955/gnj.003908.

Hassan, S.W. and El-Kassas, H.Y. (2012). Biosorption of cadmium from aqueous solutions using a local fungus *Aspergillus cristatus* (Glaucus group). Afr. J. Biotechnol., 10(72). 2276–2286. DOI: 10.5897/ajb11.3140.

Henry, R.J.; Cannon, D.C. and Winkelman, J.W. (1974). Clinical chemistry principles and techniques.Harper and Row (11th Eds). New York (NY), 1629 pp.

Hinton, D.E. and Lauren, J.L. (1990). Integrative histopathological approaches to detecting effects of environmental stressors on fishes. Am. Fish. Soc. Symp., 8: 51–66.

Ibrahim, N.M.; Eweis, E.A.; El-Beltagi, H.S. and Abdel-Mobdy, Y.E. (2012). Effect of lead acetate toxicity on experimental male albino rat. Asian Pac. J. trop. Biomed., 2(1): 41–46. DOI: 10.1016/S2221-1691(11)60187-1.

Ibrahim, S.S.; Bakery, H.H.; El-shawarby, R.M.; AbuoSalem, M.E. and Nabila, M.A. (2014). Some toxicological studies on effect of some water pollutants (heavy metals) in albino rats. Benha Vet. Med. J., 27(1): 49–61.

Iqbal, T.; Cao, M.; Zhao, Z.; Zhao, Y.; Chen, L.; Chen, T.; Li, C. and Zhou, X. (2021). Damage to the testicular structure of rats by acute oral exposure of cadmium. Int. J. Environ. Res. Public health, 18(11): 6038. DOI: 10.3390/ijerph18116038.

Iram, S.; Basharat, K. and Ahmad, I. (2013). Tole.rable analysis of the fungi of the Peri-Urban agricultural area. Pak. J. Bot. 45(S1): 475– 480

Iskandar, N.L.; Zainudin, N.A. and Tan, S.G. (2011). Tolerance and biosorption of copper (Cu) and lead (Pb) by filamentous fungi isolated from a freshwater ecosystem. J. Environ. Sci., 23(5): 824–830. DOI: 10.1016/S1001-0742(10)60475-5.

Javaid, A.; Bajwa, R.; Shafique, U. and Anwar, J. (2011). Removal of heavy metals by adsorption on *Pleurotus ostreatus*. Biomass and Bioenergy, 35(5): 1675–1682. DOI: 10.1016/j.biombioe.2010.12.035.

José, J.; Pérez, A.; Morales, j.C.; Víctor, M.; Juárez, M.; Rodríguez, I.; Cuello, M.C.; Fonseca, G.G.; Escalera, E. and Alejandra, J. M. (2019). Bioremoval of cobalt (II) from aqueous solution by three different and resistant fungal biomasses. Bioinorg. Chem. Appl., 1–8. DOI:10.1155/2019/8757149.

Joshi, P.K.; Swarup, A.; Maheshwari, S.; Kumar, R. and Singh, N. (2011). Bioremediation of heavy metals in liquid media through fungi isolated from contaminated sources. Indian J. Microbiol., 51(4): 482–487. DOI: 10.1007/s12088-011-0110-9.K.

Júnior, B.L.M.; Macedo, G.R.; Duarte, M.M.L.; Silva, E.P. and Lobato, A.K.C.L. (2003). Biosorption of cadmium using the fungus *Aspergillus niger*. Braz. J. Chem. Eng. 20(3): 229–239. DOI: 10.1590/S0104-66322003000300003.

Kara, H.; Cevik, A.; Konar, V.; Dayangac, A. and Yilmaz, M. (2007). Protective effects of antioxidants against cadmium-induced oxidative damage in rat testes. Biol. Trace Elem. Res., 120(1-3): 205–211. DOI:10.1007/s12011-007-8019-1.

Kim, K.S.; Lim, H.J.; Lim, J.S.; Son, J.Y.; Lee, J.; Lee, B.M.; Chang, S.C. and Kim, H.S. (2018). Curcumin ameliorates cadmium-induced nephrotoxicity in Sprague-Dawley rats. Food Chem. Toxicol., 114: 34–40. DOI: 10.1016/j.fct.2018.02.007

Konopka, A.; Zakharova, T.; Bischoff, M.; Oliver, L.; Nakatsu, C. and Turco, R.F. (1999). Microbial biomass and activity in lead-contaminated soil. Appl. Environ. Microbiol., 65(5): 2256–2259. DOI: 10.1128/aem.65.5.2256-2259.1999.

Kwiatkowski, N.P.; Babiker, W.M.; Merz, W.G.; Carroll, K.C. and Zhang, S.X. (2012). Evaluation of nucleic acid sequencing of the D1/D2 region of the large subunit of the 28S rDNA and the internal transcribed spacer region using SmartGene IDNS [corrected] software for identification of filamentous fungi in a clinical laboratory. J. Mol. Diagn., 14(4): 393–401. DOI: 10.1016/j.jmoldx.2012.02.004.

Lafuente, A. (2013). The hypothalamic-pituitary-gonadal axis is target of cadmium toxicity. An update of recent studies and potential therapeutic approaches. Food Chem. Toxicol., 59: 395–404. DOI: 10.1016/j.fct.2013.06.024.

Layachi, N. and Kechrid, Z. (2012). Combined protective effect of vitamin C and E on cadmium induced oxidative liver injury in rats. Afr. J. Biotechnol., 11(93): 16013–16020. DOI:10.5897/AJB12.2665.

Liu, X.R.; Wang, Y.Y.; Fan, H.R.; Wu, C.J.; Kumar, A. and Yang, L.G. (2016). Preventive effects of β-cryptoxanthin against cadmium-induced oxidative stress in the rat testis. Asian J. Androl.,18(6): 920–924. DOI:10.4103/1008-682X.173449.

López, E.E. and Vázquez, C. (2003). Tolerance and uptake of heavy metals by *Trichoderma atroviride* isolated from sludge. Chemosphere, 50(1): 137–143. DOI: 10.1016/S0045-6535(02)00485-X.

Luo, J.; Xiao, X. and Luo, Sheng-lian. (2010). Biosorption of cadmium(II) from aqueous solutions by industrial fungus *Rhizopus cohnii*. Trans. Nonferrous Met. Soc. China, 20(6): 1104–1111. DOI:10.1016/S1003-6326(09)60264-8

Mahmoud, Y.I.; Taha, A. and Soliman, S. (2018). 3-Monochloropropane-1,2-diol (alpha-chlorohydrin) disrupts spermatogenesis and causes spermatotoxicity in males of the Egyptian fruit-bat (*Rousettus aegyptiacus*). Biotech Histochem., 93(4): 293–300. DOI: 10.1080/10520295.2018.1437471.

Malik, A. (2004). Metal bioremediation through growing cells. Environ. Int., 30(2): 261–278. DOI: 10.1016/j.envint.2003.08.001.

Manguilimotan, L.C. and Bitacura, J.G. (2018). Biosorption of cadmium by filamentous fungi isolated from coastal water and sediments. J. Toxicol., 2018: 1–6. DOI: 10.1155/20 18/7170510.

Mizuno, K.; Hayashi, Y.; Kojima, Y.; Kurokawa, S.; Sasaki, S. and Kohri, K. (2007). Influence for testicular development and histological peculiarity in the testes of flutamide-induced cryptorchid rat model. Int J Urol., 14(1): 67–72. DOI: 10.1111/j.1442-2042.2006.01654.x.

Mohsenzadeh, F. and Shahrokhi, F. (2014). Biological removing of cadmium from contaminated media by fungal biomass of *Trichoderma* species. J. Environ. Health Sci. Eng., 12(1): 102. DOI: 10.1186/2052-336x-12-102.

Monachese, M.; Burton, J.P. and Reid, G. (2012). Bioremediation and tolerance of humans to heavy metals through microbial processes: a potential role for probiotics. Appl. Environ. Microbiol., 78: 6397–6404.

Mukhopadhyay, M. (2008). Role of surface properties during biosorption of copper by pretreated *Aspergillus niger* biomass. Colloids Surf. A: Physicochem. Eng. Asp., 329(1-2): 95–99. DOI: 10.1016/j.colsurfa.2008.06.052.

Murray, R.K.; Granner, D.K. and Rodwell, V.W. (2006). Harper's Illustrated Biochemistry. 27th Edition. McGraw Hill Education, New York. pp. 400–500.

Nair, A.R.; DeGheselle, O.; Smeets, K.; Kerkhove, E.V. and Cuypers, A. (2013). Cadmium induced pathologies: where is the oxidative balance lost (or not). Int. J. Mol. Sci., 14: 6116–6143. DOI: 10.3390/ijms14036116.

Pari, L. and Murugavel, P. (2005). Role of diallyltetrasulfide in ameliorating the cadmium induced biochemical changes in rats. Environ. Toxicol. Pharmacol., 20(3): 493–500. DOI: 10.1016/j.etap.2005.05.009.

Patton, C.J. and Crouch, S.R. (1977). Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. Anal. Chem., 49: 464–469. DOI:10.1021/ac50011a034.

Pavlaki, M.D.; Araújo, M.J.; Cardoso, D.N.; Silva, A.R.R.; Cruz, A.; Mendo, S.; Soares, A.M.V.M.; Calado, R. and Loureiro, S. (2016). Ecotoxicity and genotoxicity of cadmium in different marine trophic levels. Environ. Pollut., 215: 203–212. DOI: 10.1016/j.envpol.2016.05.010.

Paydin, F.; Baş, H. and Kalender, Y. (2021). Lead and cadmium induced oxidative stress in the epididymis and spleen of rats: effects of sesamol. Commagene J. Biol., 5 (1): 7–11. DOI: 10.31594/commagene.797945.

Poontawee, W.; Surapol, N. and Orawan, W. (2016). Protective effect of *Cleistocalyx nervosum var. paniala* fruit extract against oxidative renal damage caused by cadmium. Molecules, 21(133): 1–13. DOI: 10.3390/molecules21020133.

Prenika, S.; Bonita, G. and Midhat, S. (2021). Histopathological effect of arsenic in drinking water on liver and kidney of albino rat: a light microscopic study. Int. J. Sci. Res. Dent. Med. Sci., 3:166–170. DOI:10.30485/IJSRDMS.2021.313575.1213.

Razavian, S.M. and Rabie, M. (2014). Evaluation of liver biochemical parameters in manganese miners. Zahedan J. Res. Med. Sci., 16(6): 64–67.

Renu; Agarwal, M. and Singh, K. (2016). Heavy metal removal from wastewater using various adsorbents: a review. J. Water Reuse Desalin., 7(4): 387–419. DOI:10.2166/wrd.2016.104.

Ruttkay-Nedecky, B.; Nejdl, L.; Gumulec, J.; Zitka, O.; Masarik, M.; Eckschlager, T.; Stiborova, M.; Adam, V. and Kizek, R. (2013). The role of metallothionein in oxidative stress. Int. J. Mol. Sci., 14(3): 6044–6066. DOI: 10.3390/ijms14036044.

Saad, A.M. (2015). Factors affecting cobalt uptake by cobalt-trained *Mucor rouxii* NRRL 1894 biomass. Eur. J. Biotechnol. Biosci., 3(3):1–6.

Saravpreet, K.; Kuldeep, S.K. and Jasjit, K.K. (2018). Heavy metal induced histopathological alterations in liver, muscle and kidney of freshwater cyprinid, *Labeo rohita* (Hamilton). Journal of entomology and zoology studies, 6(2): 2137–2144.

Satarug, S. (2018). Dietary casdmium intake and its effects on kidneys. Toxics, 6(15): 1–23. DOI:10.3390/toxics6010015.

Sen-Gupta, R.; Kim, J.; Gomes, C.; Oh, S.; Park, J.; Im, W.B.; Seong, J.Y.; Ahn, R.S.; Kwon, H.B. and Soh, J. (2004). Effect of ascorbic acid supplementation on testicular steroidogenesis and germ cell death in cadmium-treated male rats. Mol. Cell. Endocrinol., 221(1-2): 57–66. DOI:10.1016/j.mce.2004.03.012.

Shakya, M.; Sharma, P.; Meryem, S.S.; Mahmood, Q. and Kumar, A. (2015). Heavy metal removal from industrial wastewater using fungi: uptake mechanism and biochemical aspects. J. Environ. Eng., 142(9): 1–18. DOI:10.1061/(ASCE)EE.1943-7870.0000983.

Smaoui, M.; Fatma, G.; Manel, B.; Fatma, M.A. and Abdel Fattah, E. (2000). Impact de lexposition chronique aux gaz dechappement dorigine automobile sur certains biomarqueurs touchant la fonction hormonale sexuelle mâle, la fonction renale at lhemogramme chez le rat. Pollut. Atmos., 7: 439–449.

Smith, K.R.; Klei, L.R. and Barchowsky, A. (2001). Arsenite stimulates plasma membrane NADPH oxidase in vascular endothelial cells. Am. J. Physiol. Lung Cell Mol., 280: 442–449. DOI:10.1152/ajplung.2001.280.3.L442.

Soleimani, N.; Fazli, M.M.; Ramazani, A. and Mehrasbi, M.R. (2016). Application of live, dead and dried biomasses of *Aspergillus versicolor* for cadmium biotreatment. J. Hum. Environ. Health Promot., 1(2): 87–98. DOI:10.29252/jhehp.1.2.87.

Sundararaju, S.; Manjula, A.; Kumaravel, V.; Muneeswaran, T. and Vennila, T. (2020). Biosorption of nickel ions using fungal biomass *Penicillium* sp. MRF1 for the treatment of nickel electroplating industrial effluent. Biomass Convers. Biorefin., 12(4): 1059–1068. DOI:10.1007/s13399-020-00679-0.

Taha, A. (2022). Assessment of non-target toxicity of profenofos insecticide on the aquatic bird; the white egret, *Egretta alba*. Egypt. J. Aquat. Biol. Fish., 26(2): 263–276. DOI: 10.21608/ejabf.2022.228725.

Taha, A. and Gouda, S. A. (2022). Antifertility potential of n-butanol and ethyl acetate extracts of *Penicillium oxalicum* OM282858 in male albino rats as biological control agents. J. Exp. Biol. Agric. Sci., 10(6): 1354–1365. DOI: 10.18006/2022.10 (6).1354.1365.

Taha, A. and Soliman, S. (2019). Effect of α-chlorohydrin water-bait on the fertility of captive males of the Egyptian fruit-bat (*Rousettus aegyptiacus*) and the proper time for controlling its free-ranging populations in Egypt. Egypt. J. Aquat. Biol. Fish., 23(4): 27–237. DOI:10.21608/ejabf.2019.53599.

Taha, A.; Hussien, W. and Gouda, S.A. (2023a). Bioremediation of Heavy Metals in Wastewaters: A Concise Review. Egypt. J. Aquat. Biol. Fish., 27(1): 143–166. DOI:10.21608/ejabf.2023.284415.

Taha, A.; Mohamed, S.; Mahmoud, M.A.; Saeed, E.; Fathy, M.; Mohamed, N. and Gouda, S.A. (2023b). Bioremoval of lead from polluted waters using the fungus *Talaromyces stipitatus* and its impact on male albino rats. Egypt. J. Aquat. Biol. Fish., 27(5): 429–462. DOI:10.21608/ejabf.2023.319124.

Tamura, K.; Stecher, G. and Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. National Center for Biotechnology Information, 38(7): 3022–3027. DOI: 10.1093/molbev/msab120.

Tawari-Fufeyin, P.; Ogie-Odia, E.A.; Asemota, O.C. and Balogun, A.F. (2008). Use of Amaranthus hybridus to reduce toxic effects of some heavy metals in guinea pigs (*Cavia Porcellus*) tissue. Biosci. Biotechnol. Res. Commun., 20(5): 277–281.

Tchounwou, P.B.; Yedjou, C.G.; Patlolla, A.K. and Sutton, D.J. (2012). Heavy metal toxicity and the environment. Exp. Suppl., 101: 133–164. DOI: 10.1007/978-3-7643-8340-4_6.

Thaçi, B. and Gashi, S. (2018). Reverse osmosis removal of heavy metals from wastewater effluents using biowaste materials pretreatment. Pol. J. Environ. Stud., 28(1): 337–341. DOI:10.15244/pjoes/81268.

Thapar, R.K. (2022). Evaluation of the biosorption potential of *Aspergillus flavus* biomass for removal of chromium (VI) from an aqueous solution. J. Appl. Biol. Biotechnol., 10(02): 59–67. DOI: 10.7324/jabb.2022.100208.

Thompson, J. and Bannigan, J. (2008). Cadmium: toxic effects on the reproductive system and the embryo. Reprod. Toxicol., 25(3): 304–315. DOI: 10.1016/j.reprotox.2008.02.001.

Văcar, C.L.; Covaci, E.; Chakraborty, S. Li. B.; Weindorf, D.C.; Frențiu, T.; Pârvu, M. and Podar, D. (2021). Heavy Metal-Resistant Filamentous Fungi as Potential Mercury Bioremediators. J. Fungi, 7(5): 386. DOI:10.3390/jof7050386.

Verma, D.D.; Bhatt, A. and Agrawal, P.K. (2016). In-vitro study on bioaccumulation and tolerance of heavy metals by endophytic fungi *Alternaria alternata* isolated from Cupressus torulosa D.DON. Octa J. Environ. Res., 4: 146–54.

Vimala, R. and Das, N. (2009). Biosorption of cadmium (II) and lead (II) from aqueous solutions using mushrooms: A comparative study. J. Hazard. Mater., 168: 376–382. DOI:10.1016/j.jhazmat.2009.02.062.

Wakeel, J.; Ehsan, N.; Waseem.; Akhtar, R. and Shah, S.A.H. (2020). Morphology, histopathology and hematology as biomarkers of cadmium toxicity in field rats. Iran. J. Toxicol., 14(1): 33–42. DOI:10.32598/ijt.14.1.33.

Wang, H.J.; Liu, Z.P.; Jia, X.D.; Chen, H. and Tan, Y. (2014). Endocrine disruption of cadmium in rats using the OECD enhanced TG 407 test system. Biomed. Environ. Sci., 27(12): 950–959. DOI: 10.3967/bes2014.135.

Wang, J. and Chen, C. (2006). Biosorption of heavy metals by *Saccharomyces cerevisiae*: A review. Biotechnol. Adv., 24(5): 427–451. DOI: 10.1016/j.biotechadv.2006.03.001

Whitehead, T.P.; Bevan, E.A.; Miano, L. and Leonardi, A. (1991). Defects in diagnostic kits for determination of urate in serum. Clin. Chem., 37(6): 879–881. DOI: 10.1093/clinchem/37.6.879.

Wijesekara, G.U.; Fernando, D.M.; Wijerathna, S. and Bandara, N. (2015). Environmental and occupational exposures as a cause of male infertility. Ceylon Med. J., 60: 52–56. DOI: 10.4038/cmj.v60i2.7090.

Wong, E.W. and Cheng, C.Y. (2011). Impacts of environmental toxicants on male reproductive dysfunction. Trends Pharmacol. Sci., 32(5): 290-299. DOI: 10.1016/j.tips.2011.01.001.

World Health Organization (WHO) (2000). Laboratory manual: for the examination of human semen and sperm-cervical mucus interaction. 4th Ed. Cambridge: Cambridge University Press.

Xia, L.; Xu, X.; Zhu, W.; Huang, Q. and Chen, W. (2015). A Comparative Study on the Biosorption of Cd^{2+} onto *Paecilomyces lilacinus* XLA and *Mucoromycote* sp. XLC. Int. J. Mol. Sci., 16(7): 15670–15687. DOI: 10.3390/ijms160715670.

Xu, L.C.; Sun, H.; Wang, S.Y.; Song, L.; Chang, H.C. and Wang, X.R. (2005). The roles of metallothionein on cadmium-induced testes damages in Sprague-Dawley rats. Environ. Toxicol. Pharmacol., 20(1): 83–87. DOI: 10.1016/j.etap.2004.10.008.

Yadav, N. and Khandelwal, S. (2008). Effect of Picroliv on cadmium induced testicular damage in rat. Food Chem. Toxicol., 46(2): 494–501. DOI: 10.1016/j.fct.2007.08.035.

Yang, H. and Shu, Y. (2015). Cadmium transporters in the kidney and cadmium-induced nephrotoxicity. Int. J. Mol. Sci., 16(1):1484–1494. DOI:10.3390/ijms16011484.

Yari, A.; Asadi, M.H.; Bahadoran, H.; Dashtnavard, H.; Imani, H. and Naghii, M.R. (2010). Cadmium toxicity in spermatogenesis and protective effects of L-carnitine in adult male rats. Biol. Trace Elem. Res., 137(2): 216–25. DOI: 10.1007/s12011-009-8577-5.

Yatzidis, H.L. (1987). A colorimetric method for detection of total protein. J Clinical Chemistry Journal 23: 908.

Young, D.; Joshi, A.; Huang, L.; Munk, B.; Wurzbacher, C.; Youssef, N.H.; Elshahed, M.S.; Moon, C.D.; Ochsenreither, K.; Griffith, G.W.; Callaghan, T.M.; Sczyrba, A.; Lebuhn, M. and Flad, V. (2022). Simultaneous Metabarcoding and Quantification of Neocallimastigomycetes from Environmental Samples: Insights into Community Composition and Novel Lineages. Microorganisms, 10(9): 1749. DOI:10.3390/microorganisms10091749.

Zafar, S.; Aqil, F. and Ahmed, I. (2007). Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil. Bioresour. Technol., 98(13): 2557–2561. DOI:10.1016/j.biortech.2006.09.051.

Zhao, X.; Zhang, G.; Jia, Q.; Zhao, C.; Zhou, W. and Li, W. (2011). Adsorption of Cu(II), Pb(II), Co(II), Ni(II), and Cd(II) from aqueous solution by poly(aryl ether ketone) containing pendant carboxyl groups (PEK-L): Equilibrium, kinetics, and thermodynamics. J. Chem. Eng., 171(1): 152–158. DOI:10.1016/j.cej.2011.03.080.