Biomass Conversion and Biorefinery
https://doi.org/10.1007/s13399-024-05417-4

ORIGINAL ARTICLE

Heavy metals removal potential of metal tolerant fungal biomass on leather industry effluent and assessment of treated effluent toxicity by in vitro studies

Mathiyazhagan Narayanan[1]

Received: 22 November 2023 / Revised: 23 January 2024 / Accepted: 25 January 2024

Abstract
Improper heavy metal–enriched wastewater release has emerged as an important environmental threat. The purpose of this study was to examine the possibility of using filamentous *Aspergillus flavus* and *Aspergillus niger* biomass immobilized on banana and pomegranate peels under in vitro conditions to remove pollutants from the effluent of the leather industry. The physicochemical traits were discovered to be above the allowable limits. Fortunately, both fungal species were grown together in the Potato Dextrose Agar (PDA) plate without exhibiting antagonistic traits. Remarkably, the test *A. niger* and *A. flavus* immobilized pomegranate peel and banana peel up to 82% as well as 86%, correspondingly. Under various (Set-I to Set-V) experimental conditions, the total dissolved solids (TDS), total solids (TS), biological oxygen demand (BOD), chemical oxygen demand (COD), and total suspended solids (TSS) content of treated water samples from Set-V and Set-IV were considerably reduced up to 394 and 343 mg L^{-1}, 417 and 356 mg L^{-1}, 122 and 106 mg L^{-1}, 165 and 129 mg L^{-1}, and 403 and 356 mg L^{-1}, respectively. Similarly, the experimental setup Set-V and Set-IV removed the metals such as Cr, Cu, Pd, Cd, Fe, and Zn up to 6.81 and 4.81, 3.64 and 3.44, 1.8 and 1.3, 2.4 and 1.8, 3.1 and 2.7, and 3.9 and 3.2 mg L^{-1}, respectively, from leather industry effluent. According to these results, the treated effluent had a significant reduction in toxicity as shown by the absence or decreased levels of phytotoxicity and cytotoxicity on *Vigna radiata* and *Artemia franciscana* larvae, respectively. According to these findings, *A. niger* and *A. flavus* immobilized on pomegranate and banana peels have exceptional ability to remove heavy metals and other pollutants from the leather industry effluents.

Keywords Heavy metals · Removal · Leather industry effluent · Fungal biomass · Immobilization

1 Introduction

Environmental threats posed by industrial processes and improper discharge of heavy metal–containing wastewater are growing more prevalent [1]. Leather industry has grown to lead the economic development of the nation. The Indian leather industry is currently ranked sixth in worldwide rankings, and it includes one of the country's top ten foreign currency exchange suppliers [2]. There appear to have been about 3000 tanning factories, with roughly 80% of these facilities being located in regional as well as small-scale industries [3]. A significant portion of the tanning industry is concentrated near waterways. Tannery sectors remain overwhelmingly the most hazardous owing to the wide range of chemicals (organic chemicals: tannins, phenolics, etc. inorganic: Cr, Cu, Pd, Cd, Fe, and Zn) during the processing of skins obtained from animals [4]. These chemical-enriched effluents are released into the ecosystem from the tanning facility [5].

According to the Blacksmith Institute, Vellore's waterways are among the most contaminated in the nation, having become heavily contaminated with heavy metals including Cr, Cd, Cu, Pb, and Zn [6]. These tanning techniques make use of enzymes and chemicals (H_2SO_4, NH_4Cl^-, Na_2S, and $Ca(OH)_2$). Thus, tannery wastewater from these methods exhibits a bright color and elevated levels of dissolved as well as suspended contaminants, Cr, biological oxygen demand (BOD), and chemical oxygen demand (COD). Cr(VI) toxicity represents a major source of healthcare

✉ Mathiyazhagan Narayanan
mathimicro@gmail.com

[1] Center for Research and Innovation, Department of Biotechnology, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai 602 105, Tamil Nadu, India

Published online: 24 February 2024

issues (nausea, vomiting, irritation of the skin, lungs, eyes, allergic reactions, liver and kidney toxicity) associated with tannery wastewater [7, 8]. Moreover, such colorful effluents impede light diffusion, while high COD levels lead to reduced dissolved oxygen (DO) levels throughout the aquatic ecosystem. Watering vegetation with this wastewater contaminates the soil and increases the risk of hazardous accumulation in the nutritional chain [8].

Therefore, this effluent must be cleaned before it can be properly disposed of. There are several physical (reverse osmosis, ultrafiltration, electrocoagulation, and ozonation) and chemical (ammonium salts, flocculation, and coagulation) techniques for treating tannery wastewater, all of which have advantages and disadvantages [8, 9]. For instance, these techniques have produced positive results for total suspended solids (TSS), COD removal, Cr, and turbidity, albeit potentially at the expense of increased sludge production. Similarly, the application of an electrochemical-based oxidation technique to unprocessed tannery effluent proved costly because of the excessive energy requirements that were too high and the negative effects of the effluent upon the electrodes [10]. The main disadvantage of using a membrane is the significant clogging that arises from contaminants capturing, absorbing, and coating the membrane [11]. Microbes and microbial biomasses can be exciting options for industrial wastewater remediation (e.g., bioremediation). The filamentous fungal biomass is able to remediate contaminants such as increased tannery effluent [12].

The advantages of this fungal biomass–based remediation significantly increase the removal of hazardous organic pollutants and heavy metals from effluent [13]. Enzymes such as organophosphorus hydrolases, aminohydrolases, lipase, nitrilases, hydrolases, esterases, and cutinases are produced throughout the fungal life cycle. These enzymes are also frequently detected at specific pollution levels [14]. Moreover, fungal viable or dead biomass has been recognized as a remarkably effective biosorbent substance [15] to remove harmful heavy metals from various industrial effluent and wastewater [16]. Nowadays, nearly all bioremediation studies used to treat wastewater from tanneries use a selective culture that is usually pollutant-specific and can resolve up to two pollutants, primarily Cr [17]. Effluents from tanning operations are typically complex, containing a variety of metals and organic pollutants. Furthermore, because native fungi from contaminated areas have adapted to heavy metal stress conditions, using them in metal remediation may be beneficial [18].

Using individual cells to remove various toxic heavy metals has drawbacks, including decreased cell density, decreased survival, a narrower view of bioremediation, and more challenges with effluent extraction from fungal biomass [19]. Currently, immobilization techniques are being studied to get around these drawbacks. There had been only a few studies that we were able to identify that used both viable and dead native fungus biomasses for tannery wastewater bioremediation, which could explain the study's uniqueness [20, 21]. To effectively remediate tannery wastewater with fungal biomass, an appropriate carrier material that allows metal-tolerant fungus mycelium to develop while maintaining significant wastewater remediation activities for an extended period is required [6]. Selective metal tolerant *Aspergillus* sp. biomass-based approaches to treat the leather industry effluent collected from State Industries Promotion Corporation of Tamilnadu Ltd (SIPCOT) region of Erode district of Tamil Nadu is novel report as per the author knowledge.

Fungus immobilization in/on an appropriate carrier seems to represent an effective method for accomplishing this objective. The main objectives of this investigation are as follows:

- Find sustainable and reproducible approaches to manage the leather industry effluent.
- Successfulness of approach on tannery effluent management should be analyzed especially with the toxicity factor.

In this investigation, metal-tolerant native fungus live biomass was immobilized on an appropriate carrier to remove the heavy metals from tannery effluent. Furthermore, the phyto- and cytotoxicity of treated effluent were determined by in vitro approach.

2 Materials and methods

2.1 Tannery effluent sampling and characterization

Tannery effluent was acquired from State Industries Promotion Corporation of Tamilnadu Ltd (SIPCOT) region of Erode district of Tamil Nadu, exactly from latitude: 11.37778 and longitude: 77.71324. These areas have been designated as a chronically contaminated zone by the Pollution Control Board. This has now one of the major hub's for the production of tanned leather in the country. The collected sample (100 L) (American Public Health Association (APHA), 2005) was shifted to laboratory for further studies. The physical and chemical properties like pH, total dissolved solids (TDS), total solids (TS), BOD, COD, and TSS and heavy metals such as Cr, Cu, Pd, Cd, Fe, and Zn were analyzed by adopting the protocol described in APHA, 2005 [22].

2.2 Mass cultivation of pre-identified metal tolerant fungal species

Aspergillus niger and *Aspergillus flavus*, two fungi known to be metal tolerant (pre-identified and found from metal

polluted sites [23] and reported as possess metals tolerant), were subjected in this investigation. The myceliums of these test fungal cultures were cultured separately on the Potato Dextrose Broth (PDB) with typical temperature (at 28 °C) for a week, and this process was repeated until acquired the sufficient biomass for the further experiment. Meanwhile, the antagonism (grow parallel in same medium) nature among these test isolates was investigated by typical petri plate-based assay (Supplementary Fig. 1). Briefly, these test fungal cultures were inoculated on the same PDA containing petri plates and kept for typical incubation process at 28 °C for about a week. Then, the test fungal growth patterns/nature was observed.

2.3 Immobilization of fungal biomass on suitable carrier

After obtaining the mass fungal biomass of each test fungus, each one was individually immobilized using Chau et al.'s methodology, with any necessary modifications [6]. The pomegranate peel and banana peel were used as carrier material for these test fungal cultures. Briefly, well-sliced 10 g of pomegranate peel and 10 g of banana peel were individually sterilized with 1 L of PDB in conical flask. Following sterilization, 10 mL of (each) *A. niger* and *A. flavus* spore suspensions were separately inoculated in PDB medium containing pomegranate and banana peels to promote fungal growth over the surface of carrier material as for immobilization. Then, these conical flasks were kept for incubation for 7 days at typical fungal growth temperature at 150 rpm using shaking incubator (40 °C). Later, the fungal biomass with test carrier material was harvested and subjected to dry gradually under RT and then weighed the weight of fungal biomass immobilized on carrier material.

2.4 Heavy metal removal/biosorption potential analysis on leather industry effluent

The heavy metals adsorbing/absorbing efficiency of test fungal biomass immobilized on test carrier materials was investigated through following experimental setups [6] with 1 L of leather industry effluent: Set-I: control (leather industry effluent alone), Set-II: 10 g of *A. niger* biomass, Set-III: 10 g of *A. flavus* biomass, Set-IV: 10 g of *A. flavus* and *A. niger* biomasses with pomegranate peel, Set-V: 10 g of *A. flavus* and *A. niger* biomasses with banana peel. These experimental setups were accomplished in a stirred tank model bioreactor that was additionally supplemented with 1% and 0.01% of glucose as well as NH_4NO_3, respectively. These experimental setups were maintained for 48 h at typical temperature with magnetic stirrer. Later, the heavy metal contents and other physicochemical properties of treated water were examined with typical protocol [22].

2.5 Treatment efficiency determination

Once the experiment was over, it was discovered that the treatment had significantly changed the metal and other physicochemical properties of treated leather industry effluents were subjected (including control) to phyto- and cytotoxicity study on *Vigna radiata* and *Artemia franciscana* larvae, respectively, under in vitro condition with various proportions (25:75, 50:50, 75:25, and 100:00% of treated water:tap water, respectively).

2.5.1 Phytotoxicity analysis

The method reported in Chau et al. was adopted with minor alteration to determine the phytotoxicity of treated water with needed alterations [6]. Briefly, 20 nos of *V. radiata* (0.1% of $HgCl_2$ sterilized) seeds were placed in clean filter paper containing petri plates (triplicates). This filter paper containing plates with *V. radiata* seeds was moistened by approximately 6 mL of abovementioned proportions, and separate clean tap water was applied as control. These plates were then kept for incubation using BOD incubator at typical seedling condition for about 2 weeks. The projection of *V. radiata* seedlings from each test proportions was counted and determined the germination rate.

2.5.2 Cytotoxicity analysis

The Chau et al. described methodology [6] with slight alteration as per the requirement was followed to determine the cytotoxic effects of water collected from each experimental setup with various proportions of treated water (25:75, 50:50, 75:25, and 100:00% of treated water:tap water, respectively) on *A. franciscana* larvae. Briefly, about 20 nos of *A. franciscana* larvae was incorporated in plastic container loaded with 20 mL of various proportions of water sample from each experimental setup and kept for a day at RT condition in triplicates. After a day of exposure, the larva viability was counted under microscopic observation and calculated the cytotoxic activity % (mortality %). The ocean water and 100 µg mL^{-1} of $KMnO_4$ were applied as positive and negative controls, respectively.

3 Results and discussion

3.1 Leather industry effluent quality determination

Table 1 states the physicochemical traits of effluent collected from leather industry; the results clearly state that almost of the properties were beyond the permissible limit described by the pollution control board [24]. The pH was found to be 5.5 ± 0.11; the TDS, TS, DO, BOD, COD, and TSS were

found to be 1469.5 ± 14, 3058.4 ± 30, 5.6 ± 0.5, 258 ± 18, 587 ± 22, and 1481.6 ± 34 mg L^{-1}, respectively. The heavy metals such as Cr, Cu, Pd, Cd, Fe, and Zn were found as 15.81 ± 1.1, 6.74 ± 0.49, 4.2 ± 0.21, 3.9 ± 0.17, 7.9 ± 0.38, and 8.6 ± 0.29 mg L^{-1}, respectively. These values were beyond the permissible limits of Indian standard limits. Elevated levels of harmful chemicals in untreated wastewater can lead to significant contamination of groundwater [25]. The effluents from the leather industry represent a significant ecological hazard with a significant potential for pollution of the environment. The wastewater produced by tanning and other processes contains Ca^{2+}, S^{2+}, and NH_3^-, making it naturally somewhat acidic [26]. H_2SO_4, Cr, Cl^-, $NaHCO_3$, and SO_4^{2-} were all found in scraping as well as heavy metal–enriched leather industry effluents. The process of tanning is probably responsible for these harmful substances. Many health problems have been documented in people who engage in the leather product sectors, notably skin as well as respiratory conditions brought on by this hazardous combination of chemicals [27, 28]. Both surface and groundwater are contaminated by the effluent, which can lead to respiratory disorders, kidney disease, and skin conditions like boils, itching, and diminished feeling, among other things [29, 30].

3.2 Antagonistic and mass cultivation of A. niger and A. flavus

Using PDB, *A. niger* and *A. flavus* were cultivated in large quantities, producing notable biomass yields of up to 12.3 g and 14.1 g, respectively. The results obtained from the typical PDA-based plate assay revealed that the antagonistic properties of *A. niger* and *A. flavus*. The assay's results indicate that *A. niger* and *A. flavus* grow parallel to one another in a PDA plate without exhibiting any antagonistic characteristics. This clearly indicates that *A. niger* and *A. flavus* can coexist and grow altogether on same growing medium. This may be the result of the fact that both fungal species were discovered in metal-polluted environments and have the ability to tolerate metals [23]. Metal-tolerant fungi may contain a large number of enzymes such as amylases [15, 31], laccases, catalases, lipases, xylanases, proteases, and peroxidases, which contribute to the reduction, breakdown, or detoxification of the hazardous substances in the aquatic ecosystem [32, 33]. According to this, a fungal consortium is more capable than a single individual of demonstrating an efficient bioremediation technique for metals as well as dye decomposition [33]. Furthermore, even when exposed to high concentrations of toxic substances, the combination of multiple fungal species degrades, detoxifies, and removes the heavy metals and other pollutants efficiently [34].

Table 1 Physicochemical characteristics of leather industry effluent

Parameters (mg L^{-1})	Effluent	Standard limit (mg L^{-1})
pH	5.5 ± 0.11	6.5–8.5
TDS mg L^{-1}	1469.5 ± 14	100
TS	3058.4 ± 30	-
DO	5.6 ± 0.5	-
BOD	258 ± 18	30
COD	587 ± 22	250
TSS	1481.6 ± 34	-
Cr	15.81 ± 1.1	2.00
Cu	6.74 ± 0.49	-
Pd	4.2 ± 0.21	0.05
Cd	3.9 ± 0.17	2.0
Fe	7.9 ± 0.38	4.2
Zn	8.6 ± 0.29	3.0

The values presented in the tables are mean and standard error (± SE) of triplicates

3.3 Test fungal biomass immobilization

For successful treatment of leather industry wastewater using test fungal biomass immobilized upon firm organic substances such as pomegranate peel and banana peel can able to grow while retaining significant pollutant removal from effluent for an extended period of time. Immobilization of fungal biomass on a suitable supporting substance is a promising method for achieving this goal. Organic support substances, including pomegranate peel and banana peel, exhibited significant immobilization, as well as fungal biomass, and are able to utilize the material that supports it as an alternative form of energy/nutrition. The degree of elongation between the carrier material and mycelia allowed for better test fungal growth along with improved O_2 as well as nutritive transfer effectiveness. In addition, using a carrier might offer relatively hydrodynamically steady-state circumstances, reducing hydrodynamic shear forces on such test fungi mycelia. Immobilization of *A. niger* and *A. flavus* on pomegranate peel and banana peel contained PDB media revealed that both of these species had been successfully immobilized on such solid pomegranate peel and banana peel. The active immobilization was identified by measuring pomegranate peel and banana peel after a day of colonization and then comparing it with the original weight. Remarkably, the test *A. niger* and *A. flavus* immobilized pomegranate peel and banana peel up to 82% as well as 86%, correspondingly. A research team similarly immobilized *Candida antarctica* on coconut fiber as well as demonstrated significant heavy metal adsorption/removal from polluted water and industry effluent [35]. Immobilization improves staying alive, coherence, and retention in *A.*

niger and *A. flavus*. Immobilization has begun to grow effectively in treating the industry effluent since it provides better results compared to free microbial agents and sometimes single enzyme activity [36, 37]. Immobilized fungal cultures are now preferred over traditional microbial approaches due to inherent metabolic dependability, increased productivity, sustainability, and remarkably inexpensiveness [38].

3.4 Heavy metals and other pollutant removal

The heavy metal and other pollutant removal competence of test fungal biomass and their immobilized form on pomegranate peel and banana peel solid substrate were determined by various experimental setups (Set-I to Set-V). Upon the completion of experimental condition, the physicochemical properties were analyzed and found that Set-V showed remarkable, optimistic alterations in the treated water bioreactor-based study revealed that the fungal biomass immobilized pomegranate peel and banana peel demonstrated remarkable heavy metals and other pollutants removal potential and it followed by Set-IV, Set-II, and Set-III. The most important properties such as pH and DO level were optimistically altered up to 2.3, 1.9, 1.1, 1.4, and 0.1 (Fig. 1) and 7.9, 6.0, 1.8, 2.3, and 0.3 mg L^{-1} (Fig. 2) in Set-V, Set-IV, Set-III, Set-II, and Set-I treated water, respectively. The increased DO and pH level in the treated water was considered optimistic sign of effective treatment. Other properties such as TDS, TS, BOD, COD, and TSS content of treated water sample from Set-V and Set-IV were considerably reduced up to 394 and 343 mg L^{-1}, 417 and 356 mg L^{-1}, 122 and 106 mg L^{-1}, 165 and 129 mg L^{-1}, and 403 and 356 mg L^{-1}, respectively (Fig. 3a), than other experimental setups such as Set-III, Set-II, and Set-I. Similarly, the experimental setups Set-V and Set-IV showed considerable heavy metals removal from treated leather industry wastewater sample. These experimental setups removed the metals such as Cr, Cu, Pd, Cd, Fe, and Zn up to 6.81 and 4.81, 3.64 and 3.44, 1.8 and 1.3, 2.4 and 1.8, 3.1 and 2.7, and 3.9 and 3.2 mg

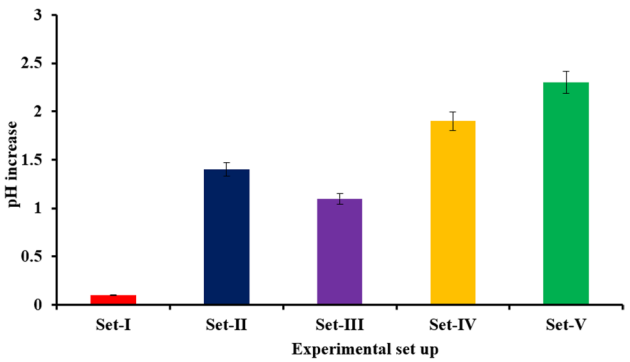

Fig. 1 Optimistic alteration in pH of treated water sample under different experimental setups

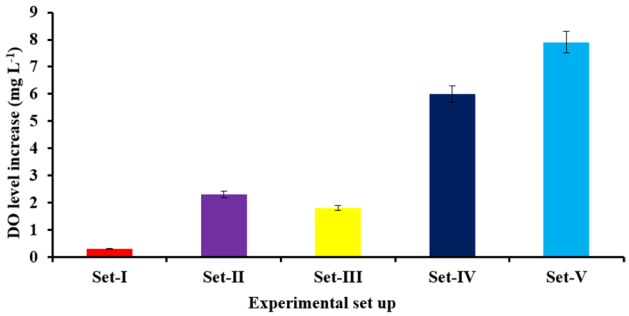

Fig. 2 Considerable raise in the DO level in treated water sample under different experimental setups

L^{-1}, respectively (Fig. 3b). These reduced values were significantly higher than the other experimental setups. On the other hand, this can be revealed as *A. niger* and *A. flavus* biomass immobilized over the surface of pomegranate peel and banana peel solid support materials effectively reduced/removed considerable quantity of metals and other pollutants from the leather industry effluent (Table 2). Acclimatization of test fungi biomass in wastewater resulted in a significant reduction in pollutants in a short period of treatment [39]. The majority of COD reduction can be credited to fungal biomass–based mineralization and the consumption of highly oxidizable components [6]. In an acidic environment, biological accumulation was discovered to serve as the main mechanism responsible for removing pollutants through immobilized *A. niger* and *A. flavus* biomass over the surface of solid support material. Chitin from fungal cell walls and chitosan both possess functional groups including amino, acetanilide, and hydroxyl groups [40]. The process in acidity condition repaired all of these functional molecules, while the contaminants' negative sulfonyl radicals became trapped to achieve equilibrium in the context of a water-based setting [41]. Hence, at acidic conditions, the biosorbent substances have a greater proclivity for absorbing additional anionic pollutants [15]. Lowering pH values favored the attraction of oxyanionic metal ions to positive charge functional elements found on *A. niger* and *A. flavus* biomass cell membranes [42]. Metal absorption occurs through a variety of mechanisms, including cell wall binding, internal accumulation, external volatilization, and precipitation [15]. Metal sorption mechanism can be categorized based on its reliance upon the fungi cell's metabolic processes which is referred to as metabolism reliant or in accordance to the site where the metal sorbed from water is discovered which is referred to as metabolism independent including external precipitation or accumulation, other mechanisms like volatilization, detoxification, and chelation occurred [6, 43]. Biosorption via inactivated fungal biomass constitutes a passive adsorption mechanism exclusively through cell surface adsorption/binding, whereas viable fungal biomass absorption is

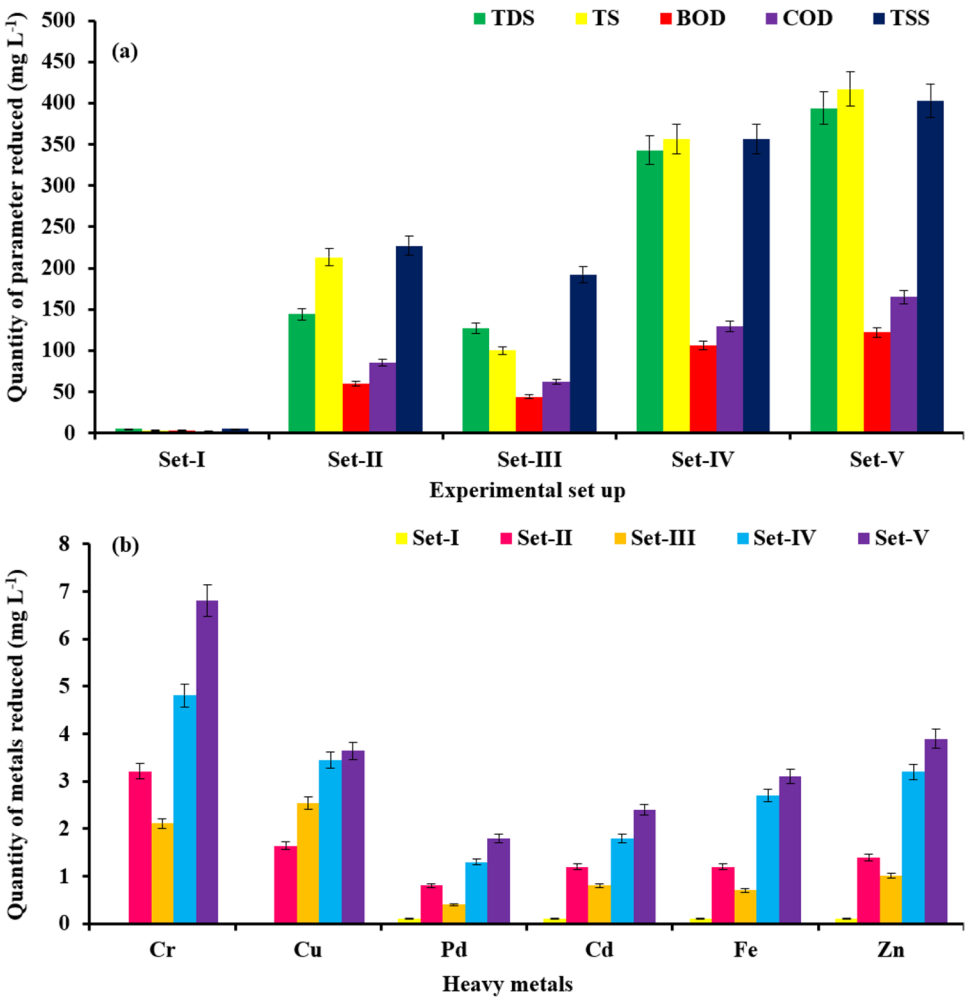

Fig. 3 Quantity of various physicochemical parameters including heavy metals removed from test water sample. **a** Parameters such as TDS, TS, BOD, COD, and TSS. **b** Heavy metals

Table 2 Physicochemical characteristics of pre- and post-treated water sample

Properties (mg L^{-1})	Pre-treated sample	Post-treated sample				
		Set-I	Set-II	Set-III	Set-IV	Set-V
pH	5.5 ± 0.11	5.4 ± 0.9	6.9 ± 0.5	6.5 ± 0.3	7.4 ± 0.6	7.8 ± 0.4
TDS	1469.5 ± 14	1464.2 ± 12	1325 ± 14	1342 ± 23	1126 ± 12	1075 ± 9
TS	3058.4 ± 30	3055 ± 28	2845 ± 36	2958 ± 16	2702 ± 21	2641 ± 7
DO	5.6 ± 0.5	5.9 ± 0.4	7.9 ± 0.2	7.4 ± 0.6	11.6 ± 13	13 ± 3
BOD	258 ± 18	255 ± 14	198 ± 10	214 ± 9	152 ± 8	136 ± 9
COD	587 ± 22	585 ± 18	502 ± 9	525 ± 4	458 ± 3	422 ± 11
TSS	1481.6 ± 34	1476 ± 21	1254 ± 16	1289 ± 13	1125 ± 13	1078 ± 13
Cr	15.81 ± 1.1	15.79 ± 0.9	12.6 ± 0.9	13.7 ± 0.8	11 ± 0.6	9 ± 0.9
Cu	6.74 ± 0.49	6.72 ± 0.5	5.1 ± 0.4	4.2 ± 0.6	3.3 ± 0.13	3.1 ± 0.5
Pd	4.2 ± 0.21	4.1 ± 0.3	3.4 ± 0.6	3.8 ± 0.62	2.9 ± 0.15	2.4 ± 0.7
Cd	3.9 ± 0.17	3.8 ± 0.7	2.7 ± 0.3	3.1 ± 0.6	2.1 ± 0.3	1.5 ± 0.6
Fe	7.9 ± 0.38	7.8 ± 0.8	6.7 ± 0.5	7.2 ± 0.17	5.2 ± 0.3	4.8 ± 0.2
Zn	8.6 ± 0.29	8.5 ± 0.7	7.2 ± 0.7	7.6 ± 0.23	5.4 ± 0.6	4.7 ± 0.6

The values presented in the tables are mean and standard error (± SE) of triplicates

a dynamic process that involves inside as well as outside metabolic processes including detoxification, bioaccumulation, chelation, and volatilization occurs [44]. Furthermore, a reduction in Cl^-, Na^+, and NO_3^- has been attributed to fungi that use these elements for proliferation [45]. Similarly, Ca^{2+} is an important intracellular signaling messenger for the development of cells as well as maintaining cytosolic accessible Ca^{2+} around 0.1 M through a variety of transportation processes located on plasma and vacuolar membranes which affect influx/efflux and vacuolar compartmentalization [46]. Dealings with Ca^{2+}-binding protein may influence cytoplasmic-free Ca^{2+}. The metal-tolerant fungal biomass immobilized on the corncob and coir progressively adsorbed the harmful substances from the leather industry effluents via the adsorption mechanisms [6, 43].

3.5 Treatment efficiency determination by toxicity analysis

3.5.1 Phytotoxicity analysis

Table 3 and Fig. 4 showed the percentage of germination and phytotoxicity, respectively, on *V. radiata* seedling exposed to water derived from each experimental setups (Set-I–Set-V). Interestingly, the Set-V and Set-IV treated water at all dilution ratio (25:75%, 50:50%, 75:25%, and 100:00%) showed 100% germination and 0% toxicity on *V. radiata* seedling. The treated water from Set-III and Set-II showed reduced germination as 70 and 60% at 25:75% and 40 and 10% at 100:00%, respectively. The phytotoxicity was not found in Set-V and Set-IV water-exposed *V. radiata* seedling [47]. Remaining experimental setup treated water samples showed certain percentage of toxicity on growth of *V. radiata* seedling (Fig. 4). Ultimately, fungal biomass immobilized pomegranate peel as well as banana peel exhibited significantly lower toxicity in leather industry effluent (treated) than non-immobilized *A. flavus* and *A. niger* biomass. Unprocessed (untreated) wastewater had no *V. radiata* seed sprouting, indicating that the wastewater that was untreated remained extremely harmful thereby completely inhibiting *V. radiata*

Table 3 *V. radiata* germination % under the exposure of treated effluents under various experimental setups

Experiment	25:75%	50:50%	75:25%	100:00%
Set-I	20 ± 1	15 ± 1	10 ± 1	0 ± 0
Set-II	60 ± 1	40 ± 2	35 ± 1	10 ± 1
Set-III	70 ± 1	65 ± 1	50 ± 1	40 ± 1
Set-IV	100 ± 1	100 ± 1	100 ± 1	100 ± 1
Set-V	100 ± 1	100 ± 2	100 ± 2	100 ± 1

The values presented in the tables are mean and standard error (± SE) of triplicates

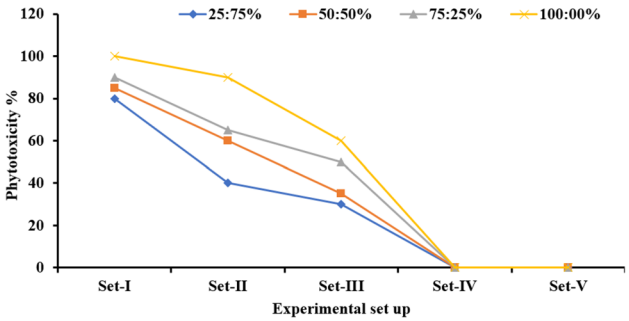

Fig. 4 Phytotoxicity (seed germination inhibition) effect on *V. radiata* exposed to treated water samples from various experimental setups

germination. A research team stated that the *Penicillium commune* and *Cladosporium perangustum* have a greater capacity for tanning facility wastewater treatment when immobilized on organic solid support substances than non-immobilized fungal biomass, reducing leather wastewater toxic effects and supporting the establishment of *Triticum aestivum* on remediated effluent [48]. Additionally, they discovered that wastewater that was untreated was harmful to seed sprouting. The detrimental impact of unprocessed leather industry effluent on crop seedlings could be attributed to its increased heavy metal content, which also promotes osmotic pressure under anaerobic environments [49]. Because under raised osmotic pressure as well as anaerobic circumstances, various physiological and biochemical mechanisms of sprouting and growth, such as solute movement, rate of respiration, as well as seed germination, as well as developmental parameters, occur [44].

3.5.2 Cytotoxicity analysis

Table 4 and Fig. 5 exhibited the viability % of *A. franciscana* larvae and cytotoxicity % which was subjected to water sample acquired from each experimental setup. Set-V as well as Set-IV treated water exhibited 100% larvae viability and 0% cytotoxicity on *A. franciscana* larvae growth regardless of all dilution ratios (25:75%, 50:50%, 75:25%, and 100:00%). Set-III as well as Set-II water that were treated exhibited lower larvae viability of 80 and 75% at 25:75% as well as 40 and 35% at 100:00%, correspondingly. The cytotoxicity of *A. franciscana* larvae growth subjected to treated water in Sets V as well as IV was not determined. The remaining experimental setup treated water samples demonstrated a particular level of cytotoxicity in *A. franciscana* larvae growth (Fig. 5). A research investigation discovered that fungal biomass–based biosorption substantially cleanses the hazardous chemicals present in leather industry effluent during biological remediation/biosorption/adsorption, as evidenced by reduced toxicity on crops and aquatic fauna [50].

Table 4 Viability of *A. franciscana* larvae exposed to treated water sample from experimental setup

Experiment	25:75%	50:50%	75:25%	100:00%
Set-I	35 ± 1	30 ± 1	20 ± 1	10 ± 0
Set-II	75 ± 1	55 ± 1	45 ± 1	35 ± 1
Set-III	80 ± 1	65 ± 1	55 ± 1	40 ± 1
Set-IV	100 ± 0	100 ± 1	95 ± 1	90 ± 1
Set-V	100 ± 1	100 ± 1	98 ± 1	93 ± 1

The values presented in the tables are mean and standard error (± SE) of triplicates

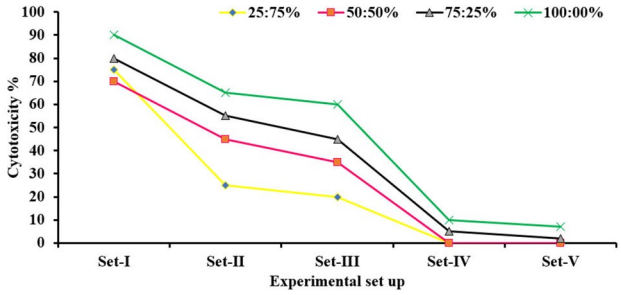

Fig. 5 Cytotoxicity effect of treated water sample from various experimental set up on *A. franciscana* larvae

Hazardous materials, like pollutants that are both inorganic and organic, are able to accumulate and diffuse throughout the food, having catastrophic effects on organisms [51]. As a result, it is critical to assess the negative effects of treated leather industry effluent as well as cereal cultivars' compatibility with agricultural irrigation [52]. The transition from dormancy to reproduction represents a critical stage in the developmental process of flora and fauna, since it governs trends in population as well as effectiveness, leading to an important ecological as well as industrial aspect [53]. Similarly, the microbes along with microbial products effectively removed the heavy metals and other pollutants from industrial effluents like leather industry effluents, leading to less cytotoxicity on *A. franciscana* larvae than untreated effluent [54]. A research investigation looked at sprouting seeds, growth, and biological characteristics to assess germination and the early growth stages that govern plant adaptations towards treated and untreated effluents [55]. Untreated harmful elements enriched effluents significantly reduced sprouting seeds and accelerated the progression of *V. radiata* as well as caused *A. franciscana* larvae fatality. Adsorbents containing active fungal biomass can remove a significant amount of harmful metals and other pollutants from effluent and thus reduce phytotoxicity by increasing chlorophyll and biomolecule content, as well as certain vital protein concentrations, when exposed to treated as well as untreated water samples [56, 57].

4 Conclusion

Leather wastewater comprises harmful elements that can kill microbes and organisms found in water, fauna, flora, and human beings, as well as induce genotoxicity, genetic modification, and cancer-causing potential. As a result, the sustainable remediation approach, methods, or agents required for effluent management must be discovered. The results show that the filamentous *A. flavus* and *A. niger* produced a significant amount of biomass and, when immobilized on organic solid support materials including pomegranate peel and banana peel, have a high ability to remove heavy metals and other pollutants from leather industry effluent (adsorption/absorption). Surprisingly, this fungal biomass immobilized on pomegranate peel and banana peel, as well as their high pollutant sorption potential from leather industry effluents, had no harmful impact on *V. radiata* and *A. franciscana* larvae. This also endorses seed growing at unique proportions, suggesting this substance decreases toxic effects (phyto- and cytotoxicity) while promoting germination of crops and larvae growth. The findings conclusively show that the filamentous *A. flavus* and *A. niger* have significant remediation capability when immobilized. To determine the viability of this strategy on leather industry effluent, a real-world evaluation is required, and this will be the investigation's future focus.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13399-024-05417-4.

Acknowledgements The author would like to thank the Center for Research and Innovations, Department of Biotechnology, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Science, Chennai-602 105, Tamil Nadu, India, for their constant support for this work.

Author contribution Mathiyazhagan Narayanan: conceptualization, investigation, writing—original draft, reviewing, and editing.

Data availability All data is available under request.

Declarations

Ethical approval Not applicable.

Competing interests The author declares no competing interests.

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