

PHYSIOLOGICAL ADAPTATION AND PHYTOREMEDIATION POTENTIAL OF A HALOPHYTE, *SESUVIUM PORTULACASTRUM* UNDER HOSPITAL WASTEWATER EXPOSURE

Tamanna Taskeen¹, Mohd Khaf², Suprasanna Penna^{3*}

¹Amity Institute of Biotechnology (AIB), Amity University, Mumbai – Pune Expressway, Bhatan, Somathne, Panvel, Mumbai, Maharashtra 410206, India

²Amity Institute of Biotechnology (AIB), Amity University, Mumbai – Pune Expressway, Bhatan, Somathne, Panvel, Mumbai, Maharashtra 410206, India

^{3*}Amity Institute of Biotechnology, Amity University, Mumbai, Maharashtra 410206, India. E-mail: psuprasanna@mum.amity.edu

ABSTRACT

Hospital wastewater (HWW) poses a serious challenge to environmental cleanup owing to its complex nature, and the presence of pharmaceutical residues, microorganisms, and cytotoxic chemicals. In this study, we assessed the phytoremediation capacity of the halophyte *Sesuvium portulacastrum* (L.) L. in a 24-day hydroponic experiment at 5% HWW concentration, extending our research on this plant species in industrial wastewater situations. Physicochemical profile showed significant reductions in parameters: BOD decreased by 56.9%, COD by 77.2%, TSS by 87.6%, and TDS by 60.10%. The plant's biochemical response was biphasic: total phenolic content increased slightly on Day 8 before declining to 8.40 mg GAE g⁻¹ by Day 24, whereas total flavonoid content progressively increased to 108.13 µg QE g⁻¹FW, indicating a shift towards flavonoid-driven antioxidant defence over time. DPPH radical scavenging activity decreased to 62.3% on Day 8 before increasing to 79.2% on Day 24, indicating physiological adaptation of *Sesuvium* plants to the wastewater exposure. Phytotoxicity testing with *Vigna radiata* seeds showed significantly better germination and seedling development in the treated HWW than untreated HWW. These laboratory-scale results indicate that *S. portulacastrum* is a low-cost, environmentally friendly alternative for hospital wastewater treatment.

KEYWORDS: Phytoremediation, halophyte, *Sesuvium portulacastrum*, hospital wastewater

1. INTRODUCTION

Hospital wastewater (HWW) is distinguished from other effluent streams by its high chemical complexity, presence of pharmaceutical residues, pathogenic bacteria, heavy metals, disinfectants, and cytotoxic medications [1,2]. Many of these including antibiotics, antineoplastic medicines, and endocrine-disrupting chemicals (EDCs), stay biologically active, *albeit* at trace quantities, making HWW extremely challenging to management [3,4,5]. When HWW is released untreated into water bodies, it promotes the antibiotic-resistant bacteria and resistance genes, a problem that is currently high on the global public health agenda [6,7]. The ecological effects are serious, as these pollutants affect the entire ecosystem including, aquatic plant reproduction, growth, and genetic integrity [8], and the genotoxic burden from chemotherapeutic medications and radiological agents adds a new dimension that is still poorly regulated in many parts of the world.

Conventional wastewater treatment facilities (WWTPs) have been in practice to handle this complexity; however, their shortcomings in eliminating pharmaceutical residues, EDCs, and resistant microorganisms from hospital effluents are also well documented [9,10,11]. Ozonation, advanced oxidation processes (AOPs), and membrane bioreactors (MBRs) provide better removal rates, but their cost and operational requirements make them unsuitable in resource-constrained situations [3]. Meanwhile, as the urban healthcare industry grows, the volume of pharmaceutical-laden effluent discharged into city water bodies, causing further selection for resistant microorganisms, also rises concurrently [12] and poses downstream risks to agriculture, drinking water sources, and aquatic ecosystems [13]. The gap between the intensity of the problem and available treatment solutions is particularly marked in the underdeveloped countries.

Phytoremediation is becoming increasingly popular as a low-cost, environmentally friendly method for treating environmental pollution [14]. Halophytes are especially promising since their innate stress tolerance allows them to thrive in the severe chemical environments of industrial and medical effluents [15,16]. Of the known phytoremediation techniques (phytoextraction, rhizofiltration, phytostabilization), rhizofiltration and phytoaccumulation have produced the most consistent outcomes in aqueous systems [17,18].

Sesuvium portulacastrum (L.) L., a perennial, salt-tolerant halophyte from the Aizoaceae family, has been intensively researched for its phytoremediation potential, particularly in heavy metal-contaminated and saline soils [19]. Its exceptional metal-accumulating ability, tolerance to high salinity, and strong growth in degraded environments make it an ideal candidate for wastewater treatment applications [20,21]. *S. portulacastrum* is often found in sandy environments such as marshes, mangroves, beaches, dunes, and salt flats. Accumulation of metals such as cadmium, lead, copper, and arsenic, as well as to tolerate oxidative stress through metabolic modifications such as antioxidant synthesis, has been well established [22,23]. Lokhande et al. [21] found that this species' antioxidant enzymes and secondary metabolites, such as

flavonoids and phenolics, were upregulated in response to metal stress. Kumawat et al. [24] investigated the bioaccumulation capacity of *Sesuvium* for the removal of heavy metals and organic contaminants from river and industrial effluent. Despite its potential, the use of *S. portulacastrum* in hospital wastewater cleanup has not been widely explored. This is one of the first studies to assess the performance of *S. portulacastrum* in hospital effluents, focusing on pharmaceuticals, pathogenic load, and other contaminants found in medical waste streams.

2. MATERIALS AND METHODS

2.1 Hospital Wastewater Collection and Plant Material

To avoid external contamination, hospital wastewater samples were collected from healthcare facilities in the Navi Mumbai region in pre-cleaned, sterile 5-litre high-density polyethylene containers. Samples were transported to the laboratory at 4°C and stored to ensure chemical integrity. The sampling approach included common hospital effluent pollutants such as medication residues, disinfectants, and possible pathogenic contaminants [25].

2.2 Characterization of Hospital Wastewater

The analysis of hospital wastewater was done to determine key physicochemical characteristics needed to estimate the initial pollutant load [26]. The analytical parameters viz., pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Dissolved Solids (TDS), and Total Suspended Solids (TSS). BOD and COD levels were calculated using conventional methods (APHA 2008). BOD was determined using the standard five-day incubation protocol. The hospital wastewater samples were diluted (1:300) with aerated BOD dilution water that contained vital nutrients and buffering agents. The samples were transferred into sealed BOD bottles with no headspace and incubated at 20°C in the dark for five days. A calibrated dissolved oxygen meter was used to measure both the initial (DO_1) and final (DO_2) values. BOD_5 ($mg\ L^{-1}$) was calculated as $(DO_1 - DO_2)/P$, where P is the decimal volumetric fraction of sample used. The characteristics of the undiluted (raw) hospital wastewater are given in Table S1. The 5% HWW treatment was prepared as 5% (v/v) raw HWW.

To calculate COD, the closed-reflux titrimetric method (APHA 5220 C) was used. Briefly, 2.5 mL of wastewater sample was combined with 1.5 mL of potassium dichromate digestion solution and 3.5 mL of sulfuric acid reagent. The mixture was digested at 150 °C in a COD reactor for two hours. After cooling, the excess dichromate was titrated with standardized ferrous ammonium sulfate (FAS) using ferroin as an indicator until the color changed sharply from blue-green to reddish-brown. A reagent blank, with distilled water in place of the sample, was processed in the same way. COD ($mg\ L^{-1}$) was calculated as $[(A - B) \times N \times 8000]/V$, where A and B are the volumes (mL) of FAS consumed by the blank and the sample, respectively, N is the normality of FAS, and V is the sample volume (mL).

Total Dissolved Solids (TDS) and Total Suspended Solids (TSS) were measured on three separate days after treatment: Day 8, Day 16, and Day 24. For each time point, treated hospital wastewater samples were collected in triplicate from experimental units labeled set 1, set 2, and set 3. On Day 8, 50 mL of wastewater was collected from each set to determine the TDS and TSS levels. On Days 16 and 24, 20 mL aliquots were removed from each replicate due to reduced water volume resulting from prolonged plant exposure and evapotranspiration. The volume remaining in each container was recorded at each sampling time.

Removal efficiency (%) of each parameter was calculated as $[(C_0 - C_t)/C_0] \times 100$, where C_0 is the concentration on Day 0 and C_t is the concentration on Day t. Because the solution volume decreased through evapotranspiration and sampling, removal was also expressed on a mass basis as $[(C_0V_0 - C_tV_t)/(C_0V_0)] \times 100$, where V_0 and V_t are the solution volumes on Day 0 and day t, respectively.

2.3 Collection and Growth of *Sesuvium portulacastrum*

Sesuvium portulacastrum plants were collected from natural halophytic zones along the coastal Mankhurd-Vashi region in Maharashtra. Healthy, uniform-sized plants in identical physiological stages were carefully removed from their natural environment. To remove adherent soil, plants were gently rinsed with distilled water and stored in plastic bags before being transported to the laboratory. Hydroponic cultivation was carried out in plastic containers under controlled conditions, using Hoagland medium enriched with 170 mM sodium chloride (NaCl) and, the plants were kept under these circumstances for 20 days to acclimatize before being exposed to hospital wastewater, following the protocol described in our previous study [60].

2.4 Experimental Setup

A hydroponic system was used to evaluate the phytoremediation effectiveness of *Sesuvium portulacastrum* when exposed to hospital effluent at various concentrations. The experimental design comprised, transparent acrylic containers for each treatment: 5%, 10%, and 15% HWW dilutions, with distilled water as the control. All treatments, including the control, were supplemented with 170 mM NaCl to mimic the plant's native halophytic environment [23]. Each container was filled with 1 litre of the appropriate solution and used to grow six uniformly sized plants. Each treatment was set up in three independent containers (biological replicates, $n = 3$). The treatment period ranged from 0 to 24 days, with sampling every 8 days to evaluate pollutant elimination and plant response dynamics across time [27].

2.5 Physiological Observations of *Sesuvium portulacastrum*

Growth parameters were examined at 8, 16, and 24-day intervals to assess plant's tolerance and adaptive response to HWW stress. The survival percentage, observable stress symptoms (chlorosis, necrosis, wilting), root and shoot development, and initiation of new roots or leaves were all recorded.

2.6 Phytochemical Analysis

Methanolic extracts were prepared to determine the phytochemical contents [28] of *Sesuvium portulacastrum*. To efficiently extract bioactive chemicals, 500 mg of fresh plant biomass was homogenized and sonicated in 10 mL of 95% methanol at 4°C for 30 minutes. To separate the particle debris, the homogenate was centrifuged at 7000 rpm for 10 minutes. The clear supernatant was collected and used for further phytochemical estimates [28].

2.6.1 Determination of Total Phenolic Content (TPC)

The Folin-Ciocalteu colorimetric assay [18] was used to measure total phenolic content (TPC) in methanolic extracts of *Sesuvium portulacastrum*, with minor modifications to Molole et al.'s method [28], as described in our previous study [60]. Fresh leaf tissues were collected from control plants (0 day; not exposed to hospital wastewater) and HWW-treated plants at 8, 16, and 24 days following exposure. To prepare the extract, 500 mg of fresh leaf tissue was homogenized in 10 mL of methanol and centrifuged at 10,000 rpm for 10 minutes at 4°C. The resulting supernatant was collected and tested for phenol content. The 200 µL of methanolic extract was mixed with 1 mL of Folin-Ciocalteu reagent (diluted 1:10 with distilled water) and incubated for 5 minutes in the dark. After adding 800 µL of 7.5% (w/v) sodium carbonate (Na_2CO_3), the mixture was incubated at room temperature for 90 minutes. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer. A calibration curve was generated using gallic acid as the standard. The total phenolic content was measured as milligrams of gallic acid equivalents per gram of fresh weight (mg GAE g⁻¹ FW).

2.6.2 Determination of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) of methanolic extracts was determined using the Molole et al. method [28], as described in our previous study [60]. Fresh leaf tissues were collected from control plants (0 day, not exposed to hospital wastewater) and HWW-treated plants at 8, 16, and 24 days following exposure. Approximately 500 mg of fresh leaf tissue was homogenized in 10 mL of methanol and centrifuged at 10,000 rpm for 10 minutes at 4 °C. The supernatant was collected and used to prepare a methanolic extract. To prepare the extract, 200 µL was combined with 600 µL of 95% ethanol, 40 µL of aluminum chloride (AlCl_3), and 40 µL of potassium acetate (1M). Then 1.12 milliliters of distilled water were added [18]. The reaction mixture was left in the dark at room temperature for 30 minutes. A UV-Vis spectrophotometer was used to measure absorbance at 415 nm, with ethanol as a blank. A calibration curve was built using quercetin as the standard. The total flavonoid concentration was reported as micrograms of quercetin equivalents per gram of fresh weight (µg QE g⁻¹ FW).

2.7 Antioxidant Assay

2.7.1 DPPH Radical Scavenging Assay

The antioxidant potential of plant methanolic extracts under hospital wastewater (HWW) treatment was evaluated using a modified DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay based on the method of Brand-Williams et al. [29] with minor modifications as described by Patil et al. [30] and in our previous study [60]. Fresh leaf tissues were collected from control (0 day) and HWW-treated plants at 8, 16, and 24 days of exposure. Methanolic extracts were prepared by homogenizing 500 mg of fresh leaf tissue in 10 mL of methanol and centrifuged at 10,000 rpm for 10 minutes at 4°C. A stock solution of DPPH (0.1 mM) was prepared in methanol and stored at -20°C. To ensure consistent radical concentration and reproducibility during testing, the stock was diluted to provide a working solution with an initial absorbance of 0.90 ± 0.02 at 517 nm. In the test, 500 µL of methanolic extract was mixed with the DPPH working solution and incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm, with methanol as a blank. A control is the absorbance of the DPPH working solution with methanol in place of extract, and A sample is the absorbance with extract. The radical scavenging capacity (RSC) was calculated using the equation: $\%RSC = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$.

2.8 Phytotoxicity

Phytotoxicity was assessed to compare the toxicity of untreated and treated (phytoremediated) HWW using *Vigna radiata* (mung bean) system, based on seed germination and seedling survival rate, as described by Mitton et al. [31]. Ten seeds were planted in sterile petri dishes covered with moist blotting paper. The treatments included distilled water (negative control), untreated HWW and treated HWW collected after 8, 16, and 24 days of phytoremediation. Each treatment was performed in triplicate. Seeds were irrigated daily with 5 mL of the appropriate treatment solution and kept under controlled laboratory conditions. The germination % was calculated upon radicle emergence, while the seedling survival rate was recorded on daily basis. Germination (%) was calculated as (number of germinated seeds/total seeds sown) $\times$ 100 and survival (%) as (number of surviving seedlings/total seeds sown) $\times$ 100. Root and shoot lengths were measured on day 7 after sowing.

2.9 Statistical Analysis

All the experiments were conducted with three independent replicates (n = 3) and data are presented as mean $\pm$ standard deviation (SD) throughout. The Shapiro-Wilk test was used to verify data normality [32]. One-way analysis of variance (ANOVA) was used to identify statistically significant differences between treatment groups for physicochemical parameters (BOD, COD, TDS, and TSS), phytochemical parameters (TPC and TFC), antioxidant activity (DPPH radical scavenging), and phytotoxicity indices (seed germination percentage, survival rate, root length, and shoot length). Tukey's honestly significant difference (HSD) post hoc test was applied for multiple pairwise comparisons where significant differences were detected ($p < 0.05$) [33]. Differences were considered significant at a 95% confidence level ($p < 0.05$). Because the same containers were sampled repeatedly over time, water-subject factor. Removal percentages of the four

water-quality parameters were compared statistically using one-way ANOVA followed by Tukey's HSD test. Given the small sample size ($n = 3$), the Shapiro-Wilk test has limited power, and residual plots were also inspected.

3. RESULTS AND DISCUSSION

3.1 Characterization of Hospital Wastewater

The collected HWW was pale yellow, turbid, and had a moderate odour, likely due to the dissolved organic matter and suspended particles, and pharmaceutical residues. Initial pollutant load was considerable, with BOD at 325 mg/L and COD at 1040 mg/L, indicating a significant organic burden. *Sesuvium portulacastrum* plants used for the phytoremediation studies were healthy, and physiologically growing. The average shoot and root lengths were 5-6 cm and 7-8 cm, respectively (Fig 1).

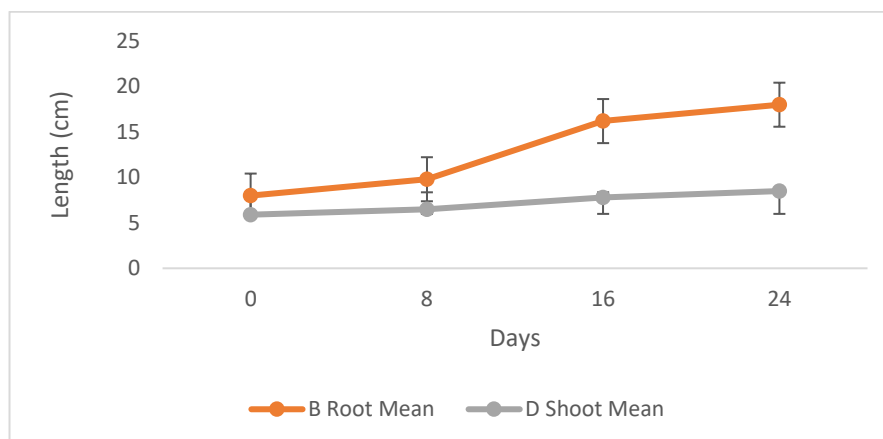


Figure 1. Root and shoot growth of *Sesuvium portulacastrum* plants over 24 days in 5% hospital wastewater.

3.2 Plant Responses to Wastewater Treatment

Concentration of HWW had a significant impact on plant survival. At 15% HWW, browning and leaf senescence was noticed by Day 8 which became more pronounced through Day 16 and 24, whereas, at 10% HWW, plants survived but displayed leaf yellowing, root darkening, and wilting from Day 4 onward, with no substantial growth. Plants remained visually healthy at 5% HWW, with no senescence, continuous root initiation, and increased shoot elongation. This concentration-dependent trend led to study the physicochemical and biological responses by using the 5% treatment. Over 24 days, the growth responses of *Sesuvium portulacastrum* plants to 5% hospital wastewater were assessed. Initial measurements on Day 1 revealed average root and shoot length, 8.0 cm and 5.9 cm, respectively. By Day 8, root and shoot lengths had increased to 9.8 cm and 6.5 cm, respectively, indicating plant acclimatization without obvious stress. By days 16 and 24, plants had grown significantly, with root lengths averaging to 16.2 cm and 18.0 cm, and shoot lengths to 7.8 cm and 8.5 cm, respectively (Fig 1). The treated HWW also displayed change in color during the study period. The HWW was dark brown on Day 8, but the HWW had lightened noticeably by Day 16 and was significantly clearer by Day 24 (Fig 2).

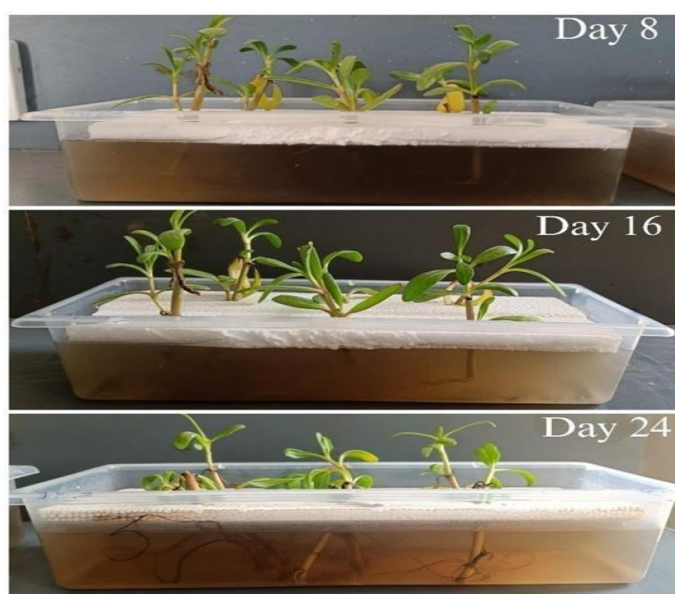


Figure 2. Decolorization of hospital wastewater upon exposure to *Sesuvium portulacastrum* plants over a period of 24 days.

3.3 Changes in the Treated HWW

In general, BOD and COD levels in the treated HWW decreased steadily from Day 0 to Day 24 (although BOD increased slightly on Day 8), with COD dropping from ~1040 mg/L to ~230 mg/L and BOD from 325 mg/L to ~140 mg/L (Fig 3 A, B).

3.3.1 Biological Oxygen Demand (BOD)

Phytoremediation with *Sesuvium portulacastrum* resulted in considerably lower BOD values ($p < 0.05$), indicating effective removal of biodegradable organic matter in hospital wastewater. Initial BOD concentrations in untreated 5% HWW were recorded at 325 mg L⁻¹, however following 8 days of treatment, BOD showed a marginal increase to 335 mg L⁻¹, likely reflecting early microbial acclimation to HWW (Fig 3A). A significant reduction was observed by Day 16, with BOD declining to 180 mg L⁻¹ ($p < 0.05$, *), representing a 44.6% decrease from the initial value (Fig 3A). By Day 24, BOD reached its lowest recorded value of approximately 140 mg L⁻¹, corresponding to a maximum removal

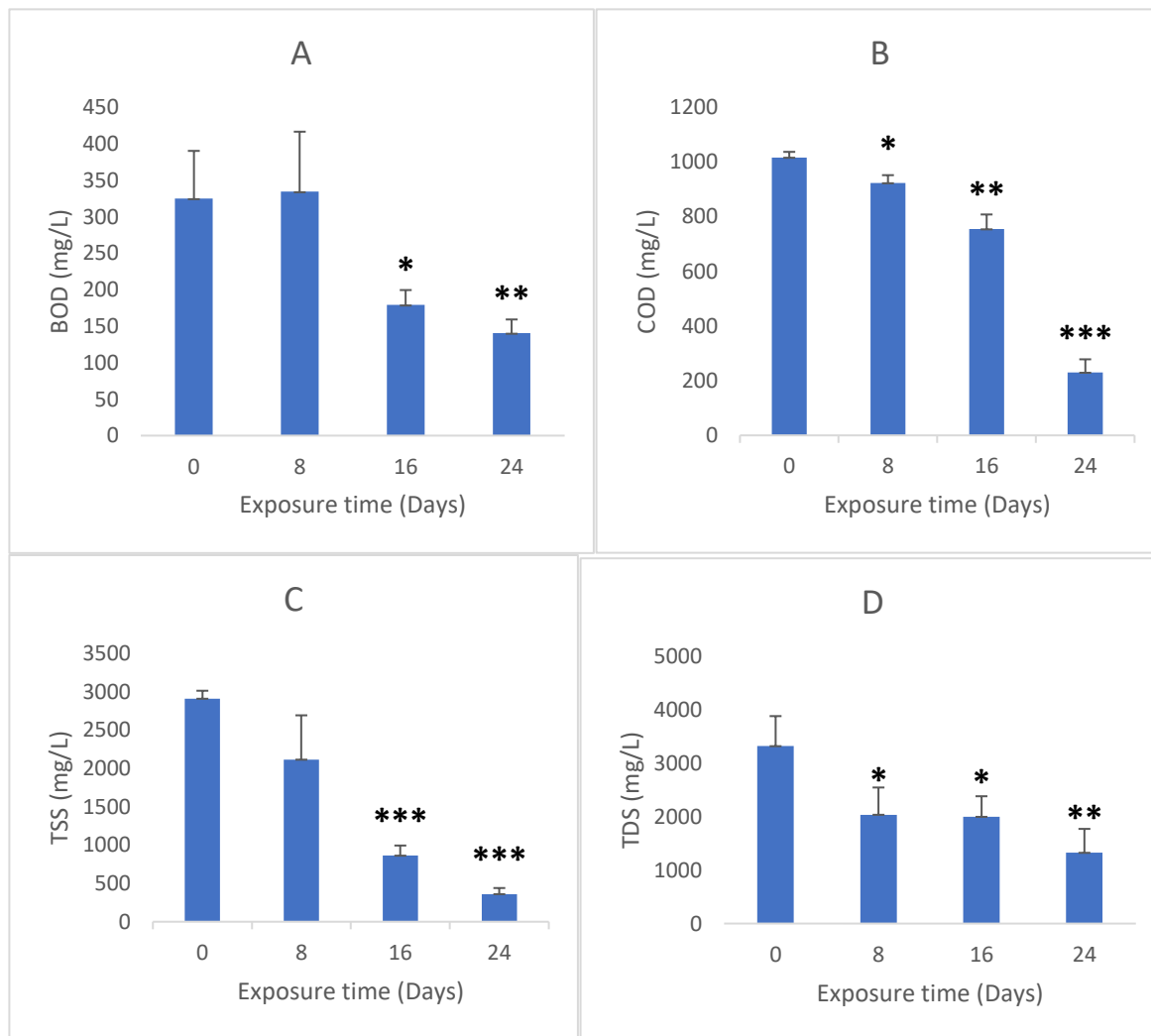


Figure 3. (A) BOD, (B) COD, (C) TSS, (D) TDS in 5% HWW treated with *S. portulacastrum* plants under exposure to HWW (0 Day, 8th day, 16th day and 24th day). Data are presented as mean $\pm$ standard deviation (SD) of three independent replicates. Asterisks (*) indicate statistically significant differences compared with the control Day 0: * $p < 0.05$, ** $p < 0.01$, * $p < 0.001$.**

Efficiency of 56.9% ($p < 0.01$, **) compared to Day 0. Huang et al. [34] observed significant reductions in biodegradable carbon fractions in floating treatment wetlands planted with *S. portulacastrum*, citing increased microbial mineralization in the rhizosphere. Similarly, Zheng et al. [35] demonstrated that *S. portulacastrum* treatments effectively reduced organic pollutants and nitrogen load in aquaculture water system. The BOD reduction observed from Day 16 onwards is consistent with the view that *S. portulacastrum* roots may support oxygen transfer, microbial colonisation and aerobic-facultative degradation of biodegradable organic matter [36]

3.3.2 Chemical Oxygen Demand (COD)

Chemical oxygen demand (COD) levels reduced significantly ($p < 0.05$) throughout the treatment period, indicating successful removal of chemically oxidizable organic matter: chemicals such as pharmaceuticals, disinfectants, and other recalcitrant substances. Initial COD was noted at approximately 1040 mg L⁻¹, however, a significant reduction was first observed at Day 8, with COD declining to approximately 930 mg L⁻¹ ($p < 0.05$, *), representing a 10.6% decrease (Fig 3B). By Day 16, COD decreased further to approximately 760 mg L⁻¹ ($p < 0.01$, **), representing a 26.9% reduction from

day 0. The most substantial reduction was recorded at Day 24, where COD declined to 230 mg L⁻¹ ($p < 0.001$, ***) (Fig 3B), representing a maximum removal efficiency of 77.9%, suggesting progressive and sustained phytoremediation activity throughout the treatment period. Huang et al. [34] found considerable decrease in dissolved organic carbon and humic-like compounds in *S. portulacastrum*-based treatment mediating the transformation of complex organic molecules. The stepwise and statistically significant COD reduction across all time points confirmed that COD reduction occurs via rhizodegradation, microbial oxidation, adsorption on root surfaces, and partial phytouptake [37].

3.3.3 Total Suspended Solids (TSS)

TSS concentrations were dramatically reduced ($p < 0.05$) after phytoremediation (Fig 3C). Untreated 5% HWW (control) had the highest TSS concentration (2908 mg L⁻¹), whereas after 8 days of treatment, TSS concentrations in treated HWW were reduced to 2113 mg L⁻¹ (27.34% reduction). Though this early-stage decline was not statistically significant, TSS levels decreased significantly to 861 mg L⁻¹ by Day 16 (Fig 3C) ($p < 0.001$, ***), corresponding to a 70.4% reduction from the initial value in TSS concentrations in untreated HWW, and reached their lowest concentration of 360 mg L⁻¹ at Day 24 ($p < 0.001$, ***), resulting in maximum removal effectiveness of 87.6%, the highest removal efficiency recorded among all physicochemical parameters in this study. The significant and progressive TSS removal across Day 16 and Day 24 is mostly due to physical filtering and trapping of dispersed particles within *S. portulacastrum*'s dense root network, combined with increased sedimentation and aggregation processes in the rhizosphere [36,38,39]. The contribution of gravitational setting could not be quantified without an unplanted control.

3.2.4 Total Dissolved Solids (TDS)

TDS removal in the HWW showed a gradual trend (Fig 3D). With initial levels at 3321 mg L⁻¹ in untreated HWW, TDS declined to 2036 mg L⁻¹ by Day 8, a statistically significant 38.7% reduction ($p < 0.05$, *). TDS showed a slight decrease to 1998 mg L⁻¹ on Day 16 ($p < 0.05$, *), which could be due to the temporary equilibrium between ion uptake by plant tissues and reversal in the medium, a pattern generally observed in rhizofiltration systems in other plants [40]. Active removal resumed at Day 24, with TDS reaching 1325 mg L⁻¹ (Fig. 3D), with a final removal of 60.1% ($p < 0.01$, **) suggesting TDS removal was sustained throughout the treatment period, which is attributed to rhizofiltration, selective ion uptake, intracellular sequestration, and rhizosphere microbial activity as the likely contributing mechanisms [40–43].

3.4 Total Phenolic and Flavonoid Content

Sesuvium portulacastrum exhibited a significant metabolic response to hospital wastewater exposure, characterized by an altered secondary metabolite profile. Compared to 12.13 mg GAE g⁻¹FW TPC of untreated (control) plants, exposure to 5% HWW resulted in a slight increase in TPC to 12.85 mg GAE g⁻¹ FW on Day 8 ($p < 0.01$, **), indicating early activation of antioxidant defence mechanisms. Prolonged exposure led to a progressive decrease in TPC to 10.21 mg GAE g⁻¹ FW on Day 16 ($p < 0.001$, ***) and reached 8.40 mg GAE g⁻¹ FW on Day 24, representing a significant reduction of approximately 30.8% compared to the control ($p < 0.001$, ***). This significant decline suggests that phenolic compounds are continuously upregulated for anti-oxidant response under prolonged oxidative stress imposed by hospital wastewater [44].

On the other hand, in case of flavonoids, the TFC in control plants was 67.46 µg QE g⁻¹FW, and it increased at every time point in treated plants (79.84 and 92.76 µg QE g⁻¹FW on Day 8 and 16; $p < 0.01$ and $p < 0.001$, respectively), reaching 108.13 µg QE g⁻¹FW by Day 24, a 60.3% increase over control ($p < 0.001$, ***) (Figure 4). Unlike phenolics, flavonoids showed an increasing trend, suggesting a metabolic shift toward more stable antioxidant compounds as a longer-term protective strategy [45,46]. Taken together, the TPC and TFC data show a biphasic response: rapid phenolic deployment in the early stress phase, followed by flavonoid accumulation as the dominant long-term protective mechanism [47]. This pattern is consistent with stress adaptation mechanisms documented in halophytes [48–54], and the continued divergence between TPC and TFC pathways over the 24 days highlights the role of flavonoids as indicators of sustained metabolic adaptation [53,54]. From a practical standpoint, the plant's sustained biochemical activity throughout the treatment period is consistent with its phytoremediation potential [55].

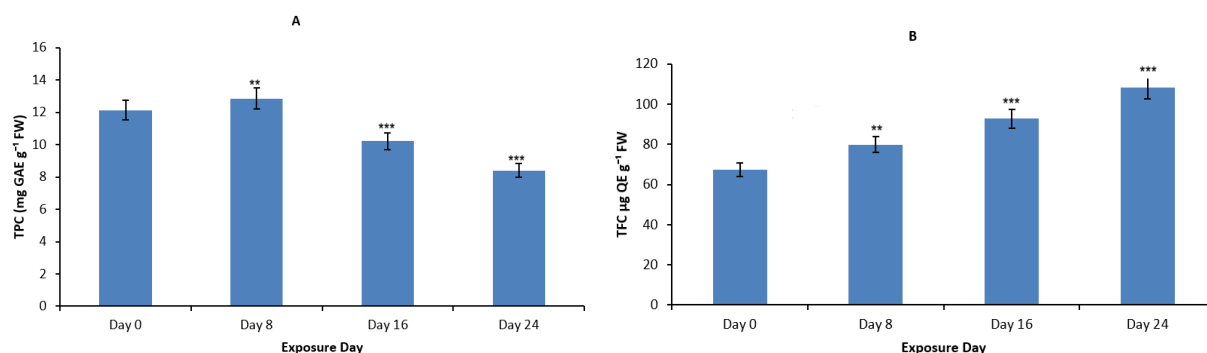


Figure 4. A. Total phenolic content (TPC) of *Sesuvium portulacastrum* plants exposed to 5% hospital wastewater for 24 days. Values are expressed as mean $\pm$ SD ($n = 3$). * $p < 0.001$ compared with the Day 0 control. B. Total flavonoid content (TFC) of *Sesuvium portulacastrum* plants exposed to 5% hospital wastewater for 24 days. Values are expressed as mean $\pm$ SD ($n = 3$). *** $p < 0.001$ compared with the Day 0 control.**

3.4 Antioxidant Activity

3.4.1 DPPH Radical Scavenging Activity

The results on the DPPH Radical Scavenging Activity showed a higher DPPH radical scavenging activity of 85.5% RSC, reflecting the elevated constitutive antioxidant capacity typical of halophytes adapted to saline, oxidatively stressful environments [53]. When exposed to HWW, %RSC dropped sharply to 62.3% by Day 8 ($p < 0.001$, ***), a 27.1% decline that indicates the plant's baseline antioxidant responses to the pharmaceutical and xenobiotic load of the wastewater (Figure 5) [56]. From Day 8 onward, %RSC showed an increase of 74.8% by Day 16 ($p < 0.05$, *) and 79.2% by Day 24 ($p < 0.05$, *), recovering approximately by 72.7% of the control. The upward trend from Day 8 to Day 24 suggests an adaptive response of the plants to the stress imposed by HWW [57,58] and that flavonoid biosynthesis may be the primary mechanism of the restored radical scavenging capacity [45,46,53].

$\mu\text{g QE g}^{-1}\text{FW}$

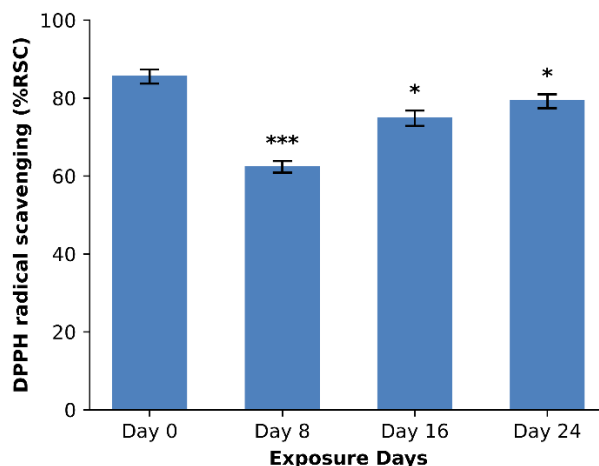


Figure 5. DPPH radical scavenging activity (%RSC) of *Sesuvium portulacastrum* leaves exposed to 5% hospital wastewater for 0, 8, 16 and 24 days. Values are expressed as mean $\pm$ SD ($n = 3$). Asterisks indicate significant differences compared with Day 0 (one-way ANOVA followed by Tukey's HSD test): * $p < 0.05$, *** $p < 0.001$.

3.5 Phytotoxicity Studies

A phytotoxicity assay was conducted using mung bean (*Vigna radiata*) seeds to assess the impact of phytoremediated HWW on plant development. Seeds irrigated with untreated HWW displayed delayed germination, stunted primary root formation, and weak seedling development, with 70% germination and survival (Table 1). Morphological observations revealed chlorosis and suppressed shoot elongation, suggesting possible disruption of cell division, membrane integrity, and nutrient uptake by the pharmaceutical contaminants in HWW [44,52]. Seeds irrigated with treated HWW at Day 8 showed 100% germination and survival, with normal root and shoot emergence and no visible stress symptoms (Figure 6 A-C). Germination rates remained at 90% even at Days 16 and 24, with robust seedling growth suggesting the physicochemical reductions mediated by the halophyte *S. portulacastrum* through detoxification of specific analytes may have resulted in less toxicity to the tested *Vigna* seeds [38,59]. The results confirm findings from other halophyte-based phytoremediation studies [34,43] and constructed wetland systems treating industrial and hospital effluents [26,31]. Taken together, the germination data suggest that wastewater treated for 8 days by *S. portulacastrum* may find reuse applications, however, field-scale studies will substantiate the results obtained in the study.

Table 1. Phytotoxicity study parameters of mung bean (*Vigna radiata*).

Parameters	Untreated HWW (5%)	HWW Treatment period		
		Day 8	Day 16	Day 24
Root Length (cm)	0.50 $\pm$ 0.10	1.50 $\pm$ 0.10	2.00 $\pm$ 0.10	6.00 $\pm$ 0.10
Shoot Length (cm)	2.00 $\pm$ 0.10	10.00 $\pm$ 0.10	11.03 $\pm$ 0.15	13.00 $\pm$ 0.10
Survival (%)	70.0 $\pm$ 1.0	80.0 $\pm$ 1.0	80.0 $\pm$ 2.0	85.0 $\pm$ 1.0
Germination (%)	70.0 $\pm$ 1.0	80.0 $\pm$ 1.0	80.0 $\pm$ 2.0	85.0 $\pm$ 1.0

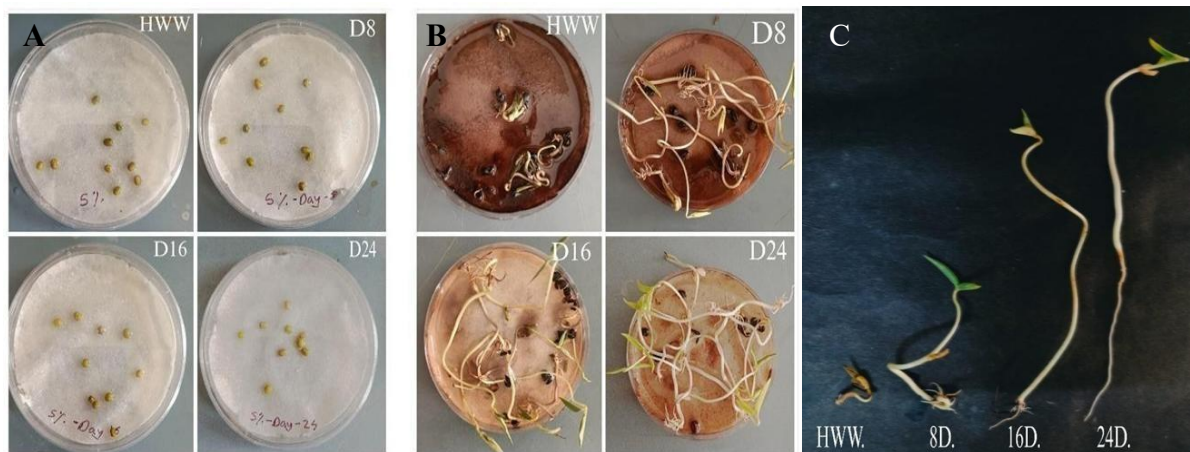


Figure 6. Phytotoxicity effect on seed germination of mung bean (*Vigna radiata*) : A- Day 1 of germination, B- Day 7 of germination, C- seedling growth of HWW and treated- HWW grown single seedlings over 8-24 days. Progressive increase in root and shoot length observed, with maximum growth at 24 days of treatment.

4. CONCLUSION

This study has highlighted the potential of *Sesuvium portulacastrum*, a halophyte for application in industrial and heavy-metal remediation contexts, for phytoremediation of hospital wastewater. The results showed that over 24 days of hydroponic treatment at 5% HWW, BOD decreased by 56.9%, COD by 77.9%, TSS by 87.6%, and TDS by 60.1%. Higher removal of TSS reflected the physical filtration capacity of the plant's dense root network. Biochemically, phenolic accumulation and sustained flavonoid accumulation and DPPH radical scavenging capacity from Day 8 to Day 24 indicated better growth and development of plants in an active metabolic state for phytoremediation. The phytotoxicity data based on mung bean germination assay suggested the treated effluent may be suitable for reuse applications. Further work is needed on longer treatment durations, higher plant densities, and integration with large field experiments. Nonetheless, the study offers a viable applied strategy for hospital wastewater management in developing countries aiming at environmental sustainability.

Authors' Contributions

Conceptualization: T.T. and S.P.; Methodology: T.T. and M.K.; Investigation: T.T. and M.K.; Writing – original draft preparation: T.T.; Writing – review and editing: S.P. All authors have read and agreed to the published version of the manuscript.

Funding

No funding was received for this study.

Ethical Approvals

Institutional Review Board Statement: Not applicable. Informed Consent Statement: Not applicable.

Data Availability Statement

The original data presented in this study are available from the corresponding author upon reasonable request.

Acknowledgements

The authors would like to thank Prof. A.W. Santosh Kumar, Hon'ble Vice Chancellor, Amity University Maharashtra, Mumbai, for support and for providing facilities in carrying out the research. The authors used ChatGPT (OpenAI) and Claude (Anthropic) solely for language editing and improving the readability of the manuscript. All scientific content, data interpretation, and conclusions are the authors' own. The authors take full responsibility for the accuracy and integrity of the work.

Conflicts Of Interest

The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

HWW	Hospital Wastewater
THWW	Treated Hospital Wastewater
COD	Chemical Oxygen Demand
BOD	Biochemical Oxygen Demand
TS	Total Solids
TSS	Total Suspended Solids
TDS	Total Dissolved Solids

DMSO	Dimethyl Sulfoxide
TPC	Total Phenolic Content
TFC	Total Flavonoid Content
DNSA	Dinitro Salicylic Acid

REFERENCES

1. Verlicchi P, Al Aukidy M, Zambello E. Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment— A review. *Sci Total Environ.* 2012;429:123-155.
2. Belle G, Moodley B, Moodley R, Omotola EO, Truter C, Olatunji O, et al. Occurrence and detection of selected pharmaceuticals of emerging concern: Potential risks for aquatic ecosystems and human health. *Discov Appl Sci.* 2025;7:1078.
3. Luo Y, Guo W, Ngo HH, Nghiem LD, Hai FI, Zhang J, Liang S, Wang XC. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Sci Total Environ.* 2014;473-474:619-641.
4. Emmanuel E, Perrodin Y, Keck G, Blanchard JM, Vermande P. Ecotoxicological risk assessment of hospital wastewater: A proposed framework for raw effluents discharging into urban sewer network. *J Hazard Mater.* 2005;117(1):1-11.
5. Khan AHA, Barros R. Pharmaceuticals in water: Risks to aquatic life and remediation strategies. *Hydrobiology.* 2023;2(2):395-409.
6. Michael I, Rizzo L, McArdell CS, Manaia CM, Merlin C, Schwartz T, et al. Urban wastewater treatment plants as hotspots for antibiotic-resistant bacteria and genes spread into the environment: A review. *Sci Total Environ.* 2013;447:345-360.
7. Chaber-Jarlachowicz P, Gworek B, Kalinowski R. Removal efficiency of pharmaceuticals during the wastewater treatment process: Emission and environmental risk assessment. *PLoS One.* 2025;20(9):e0331211.
8. Kümmerer K. The presence of pharmaceuticals in the environment due to human use: Present knowledge and future challenges. *J Environ Manage.* 2009;90(8):2354-2366.
9. Rivera-Utrilla J, Sánchez-Polo M, Ferro-García MA, Prados-Joya G, Ocampo-Pérez R. Pharmaceuticals as emerging contaminants and their removal from water: A review. *Chemosphere.* 2013;93(7):1268-1287.
10. Matesun J, Petrik L, Musvoto E, Ayinde W, Ikumi D. Limitations of wastewater treatment plants in removing trace anthropogenic biomarkers and future directions: A review. *Ecotoxicol Environ Saf.* 2024;281:116610.
11. aus der Beek T, Weber FA, Bergmann A, Hickmann S, Ebert I, Hein A, et al. Pharmaceuticals in the environment— Global occurrences and perspectives. *Environ Toxicol Chem.* 2016;35(4):823-835.
12. Patel M, Kumar R, Kishor K, Mlsna T, Pittman CU Jr, Mohan D. Pharmaceuticals of emerging concern in aquatic systems: Chemistry, occurrence, effects, and removal methods. *Chem Rev.* 2019;119(6):3510-3673.
13. Kumar K, Gupta SC, Baidoo SK, Chander Y, Rosen CJ. Antibiotic uptake by plants from soil fertilized with animal manure. *J Environ Qual.* 2005;34(6):2082-2085.
14. Kafle A, Timilsina A, Gautam A, Adhikari A, Bhattarai A, Aryal N. Phytoremediation: Mechanisms, plant selection and enhancement by natural and synthetic agents. *Environ Adv.* 2022;8:100203.
15. Zhang S, Yin X, Arif M, Chen S, Ma M, Zhu K, et al. Strategy matters: Phytoremediation potential of native halophytes is jointly associated with their distinct salt tolerances. *J Clean Prod.* 2023;425:139060.
16. Shelke DB, Chambhare MR, Sonawane HB, Islam NF, Patowary R, Das MR, et al. Synergistic approaches in halophyte-microbe interactions: Mitigating soil salinity and industrial contaminants for sustainable agriculture. *Discov Life.* 2025;55(1):11.
17. Alsafran M, Usman K, Ahmed B, Rizwan M, Saleem MH, Al Jabri H. Understanding the phytoremediation mechanisms of potentially toxic elements: A proteomic overview of recent advances. *Front Plant Sci.* 2022;13:881242.
18. Sharma JK, Kumar N, Singh NP, Santal AR. Phytoremediation technologies and their mechanism for removal of heavy metal from contaminated soil: An approach for a sustainable environment. *Front Plant Sci.* 2023;14:1076876.
19. Lokhande VH., Nikam TD., Suprasanna P. *Sesuvium portulacastrum* (L.) L. a promising halophyte: cultivation, utilization and distribution in India. *Gene. Res. Crop Evol.* 2009; 56:741–747. doi: 10.1007/s10722-009-9435-1.
20. Lokhande VH, Suprasanna P. Prospects of halophytes in understanding and managing abiotic stress tolerance. In: Ahmad P, Prasad MNV, editors. *Environmental Adaptations and Stress Tolerance of Plants in the Era of Climate Change.* New York: Springer; 2012. p. 29-56.
21. Lokhande VH, Gor BK, Desai NS, Nikam TD, Suprasanna P. *Sesuvium portulacastrum*, a plant for drought, salt stress, sand fixation, food and phytoremediation: A review. *Agron Sustain Dev.* 2013;33(2):329-348.
22. Lokhande VH, Srivastava S, Patade VY, Dwivedi S, Tripathi RD, Nikam TD, Suprasanna P. Investigation of arsenic accumulation and tolerance potential of *Sesuvium portulacastrum* (L.) L. *Chemosphere.* 2011;82(4):529-534.
23. Nikalje GC, Suprasanna P. Coping with metal toxicity—cues from halophytes. *Front Plant Sci.* 2018;9:777.
24. Kumawat AK, Vaish S, Pathak B. Phytoremediation efficacy of *Sesuvium portulacastrum* L. in mitigating industrial effluents and heavy metal contamination. *Environ Sci Pollut Res.* 2025;32(13):8340-8357.

25. Majumder A, Gupta AK, Ghosal PS, Varma M. A review on hospital wastewater treatment: A special emphasis on occurrence and removal of pharmaceutically active compounds, resistant microorganisms, and SARS-CoV-2. *J Environ Chem Eng.* 2021;9(2):104812.
26. Watharkar AD, Khandare RV, Waghmare PR, Jagdale AD, Govindwar SP, Jadhav JP. Treatment of textile effluent in a developed phytoreactor with immobilized bacterial augmentation and subsequent toxicity studies on *Etheostoma olmstedi* fish. *J Hazard Mater.* 2015;283:698-704.
27. Uddin MM, Chen Z, Xu F, Huang L. Physiological and cellular ultrastructural responses of *Sesuvium portulacastrum* under Cd stress grown hydroponically. *Plants.* 2023;12(19):3381.
28. Molole GJ, Gure A, Abdissa N. Determination of total phenolic content and antioxidant activity of *Commiphora mollis* resin. *BMC Chem.* 2022;16(1):48.
29. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci Technol.* 1995;28(1):25-30.
30. Patil R, Aware C, Shinde K, Ghanekar RK, Vyavahare G, Bapat V, et al. Anti-inflammatory and antioxidant properties of *Mucuna sanjappae* seeds in rat model and *in vitro* assays. *Biomed Pharmacol J.* 2024;17:713-724.
31. Mitton FM, Gonzalez M, Monserrat JM, Miglioranza KSB. Potential use of edible crops in the phytoremediation of endosulfan residues in soil. *Chemosphere.* 2016;148:300-306.
32. Ghasemi A, Zahediasl S. Normality tests for statistical analysis: A guide for non-statisticians. *Int J Endocrinol Metab.* 2012;10(2):486-489.
33. Zar JH. *Biostatistical Analysis.* 5th ed. Upper Saddle River, NJ: Pearson Prentice Hall; 2010.
34. Huang L, Zhuo J, Guo W, Spencer RGM, Zhang Z, Xu J. Tracing organic matter removal in polluted coastal waters via floating bed phytoremediation. *Mar Pollut Bull.* 2013;71(1-2):74-82.
35. Zheng S, Wu M, Zhan L, Lin Y, Yang M, Zheng H, et al. Effects of *Sesuvium portulacastrum* floating treatment wetlands on nitrogen removal and carbon sequestration in aquaculture water. *Water.* 2024;16(23):3472.
36. Vymazal J. Constructed wetlands for treatment of industrial wastewater: A review. *Ecol Eng.* 2014;73:724-751.
37. Stefanakis AI. The role of constructed wetlands as green infrastructure for sustainable urban water management. *Sustainability.* 2019;11(24):6981.
38. Calheiros CSC, Rangel AOSS, Castro PML. Treatment of industrial wastewater with constructed wetlands planted with *Typha latifolia* and *Phragmites australis*. *Bioresour Technol.* 2009;100(13):3205-3213 .
39. Vymazal J. Constructed wetlands for wastewater treatment. *Water.* 2010;2(3):530-549.
40. Dushenkov V, Kumar P.B.A.N, Motto H, Raskin I; Rhizofiltration: The Use of Plants to Remove Heavy Metals from Aqueous Streams. *Environ. Sci. Technol.* 1995; 29 (5): 1239–1245. <https://doi.org/10.1021/es00005a015>.
41. Manousaki E, Kalogerakis N. Halophytes Present New Opportunities in Phytoremediation of Heavy Metals and Saline Soils. *Ind. Eng. Chem. Res.* 2011; 50 (2): 656–660. <https://doi.org/10.1021/ie100270x>.
42. Saiyood S, Vangnai AS, Inthorn D, Thiravetyan P. Treatment of total dissolved solids from industrial wastewater using halophytes. *Water Air Soil Pollut.* 2012;223: 4865–4873 .
43. Manousaki E, Kalogerakis N. Halophytes—An Emerging Trend in Phytoremediation, *International Journal of Phytoremediation*, 2011. 13:959-969, doi: 10.1080/15226514.2010.532241.
44. Emmanuel E, Hanna K, Bazin C, Keck G, Clément B, Perrodin Y. Fate of glutaraldehyde in hospital wastewater and combined effects of glutaraldehyde and surfactants on aquatic organisms. *Environ Int.* 2005;31(3):399-406.
45. Tattini M, Galardi C, Pinelli P, Massai R, Remorini D, Agati G. Differential accumulation of flavonoids and hydroxycinnamates in leaves of *Ligustrum vulgare* under excess light and drought stress. *New Phytol.* 2004;163(3):547-561.
46. Nakabayashi R, Yonekura-Sakakibara K, Urano K, Suzuki M, Yamada Y, Nishizawa T, et al. Enhancement of oxidative and drought tolerance in *Arabidopsis* by overaccumulation of antioxidant flavonoids. *Plant J.* 2014;77(3):367-379.
47. Løvdaal T, Olsen KM, Slimestad R, Verheul M, Lillo C. Synergetic effects of nitrogen depletion, temperature, and light on the content of phenolic compounds and gene expression in leaves of tomato. *Phytochemistry.* 2010;71(5-6):605-613.
48. Michalak A. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Pol J Environ Stud.* 2006;15(4):523-530.
49. Kapoor, D., Singh, S., Kumar, V., Romero Carrillo, R., Singh, Jr. (2019). Antioxidant enzymes regulation in plants in reference to reactive oxygen species (ROS) and reactive nitrogen species (RNS). *Plant Gene.* 19. 10.1016/j.plgene.2019.100182.
50. Pilon-Smits EAH. Phytoremediation. *Annu Rev Plant Biol.* 2005;56:15-39.
51. Agati G, Azzarello S, Pollastri S, Tattini M. Flavonoids as antioxidants in plants: Location and functional significance. *Plant Sci.* 2012;196:67-76.
52. Cesco S, Mimmo T, Tonon G, Tomasi N, Pinton R, Terzano R, et al. Plant-borne flavonoids released into the rhizosphere: Impact on soil bio-activities related to plant nutrition. *Biol Fertil Soils.* 2012;48(2):123-149.
53. Flowers TJ, Colmer TD. Salinity tolerance in halophytes. *New Phytol.* 2008;179(4):945-963.
54. Slama I, Abdelly C, Bouchereau A, Flowers T, Savoure A. Diversity, distribution and roles of osmoprotective compounds accumulated in halophytes under abiotic stress. *Ann Bot.* 2015;115(3):433-447.
55. Ali H, Khan E, Sajad MA. Phytoremediation of heavy metals — concepts and applications. *Chemosphere.* 2013;91(7):869-881.

56. Peng FQ, Ying GG, Yang B, Liu S, Lai HJ, Liu YS, et al. Biotransformation of progesterone and norgestrel by two freshwater microalgae *Scenedesmus obliquus* and *Chlorella pyrenoidosa*: Transformation kinetics and product identification. *Chemosphere*. 2014;95:581-588.
57. Apel K, Hirt H. Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *Annu Rev Plant Biol*. 2004;55:373-399.
58. Mittler R. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci*. 2002;7(9):405-410.
59. Vymazal, J. Plants used in constructed wetlands with horizontal subsurface flow: a review. *Hydrobiologia* 674, 133–156 2011; <https://doi.org/10.1007/s10750-011-0738-9>.
60. Taskeen T, Patil SC, Patil R, Nikalje GC, Penna S. Evaluation of *Sesuvium portulacastrum* (L.) L. as a halophytic candidate for the phytoremediation of industrial wastewater. *Sustainability*. 2026;18(11):5439. doi:10.3390/su18115439.