Removal of pentachlorophenol from contaminated wastewater using phytoremediation and bioaugmentation processes

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ABSTRACT

The phytoremediation procedure was conducted by Lemna gibba (L) and Typha angustifolia (T) and the bioaugmentation procedure used P. putida HM627618. The ability of the selected P. putida HM627618 to tolerate and remove PCP (200 mg L⁻¹) was measured by high performance liquid chromatography analysis and optical density at 600 nm. Five different experiments were conducted in secondary treated wastewater for PCP testing removal (100 mg L⁻¹) including two phytoremediation assays (T + PCP; L + PCP), three bioaugmentation-phytoremediation assays (T + B + PCP; L + B + PCP; L + T + B + PCP) and a negative control assay with PCP. Various analytical parameters were determined in this study such as bacterial count, chlorophylls a and b, COD, pH and PCP content. The main results showed that the average PCP removal by P. putida HM627618 was around 87.5% after 7 days of incubation, and 88% of PCP removal was achieved by treatment (T + B) after 9 days. During these experiments, pH, COD and chloride content showed a net increase in all treatments. The chlorophylls a and b in case of (T) and (L) Chlorophylls a and b for T and L phytoremediation showed a decrease with a value less than 10 μg/mg of fresh material after 20 days of cultivation.

Key words: Lemna gibba, pentachlorophenol, Pseudomonas putida, Typha angustifolia, wastewater

HIGHLIGHT

- The use of macrophytes with two categories: aquatic fixed and floating models to treat polychlorinated pollutants from wastewater. This study evaluated the efficiency of Lemna gibba and Typha angustifolia to remove pentachlorophenol from aqueous solution further, and the combined process of phytoremediation and bioaugmentation efficacy in PCP removal by the plant.
INTRODUCTION

The intensification of the accumulation of micropollutants in treated wastewater has generated and activated many investigations concerning their removal and their biological treatment (Werheni et al. 2021). Recently, the use of aquatic plants in wastewater treatment is an emerging pollutant removal method. Studies by Obinna & Enyoh (2020) and Werheni et al. (2021) have shown the effectiveness of using plants in engineered wetlands at pilot scale or hydroponic facilities for wastewater treatment. The ability of plants for soil or water bioremediation depends on the general absorption mechanism of each species, depending on their genetic, physiological, anatomical and morphological characteristics (Rahman & Hasegawa 2011; Nguyen et al. 2020). In this situation, plants often used for phytoremediation should show rapid growth with high biomass development, deep roots and easier leaves, fruits, etc. to harvest (Garcinuno et al. 2006). Various species of floating plants such as *Lemna* sp. have been examined for metals and organic micropollutants removal through a massive filtration process (Priya et al. 2012; Chaudhuri et al. 2014; Ennabili et al. 2019). But, the ability of *Lemna* to degrade PCP is not yet well studied. The study of Ennabili et al. (2019) and Sasmaz et al. (2015) reported that *Lemna* sp. showed good performance concerning pesticides removal and no long-term toxicity detection. Also, the aquatic plant of *T. angustifolia* showed the advantage of growing and mounting under various climatic conditions; these properties and characteristics were exploited in biofilters for lakes, lagoons and estuaries water protection (Milam 2004; Werheni et al. 2020). These kinds of macrophyte showed the required characteristics of phytoremediation because of their rapid growth, easy spreading and harvesting. In addition, it has been revealed that *T. angustifolia* has the ability to tolerate and remove various xenobiotic compounds, e.g., atrazine, metalaxyl, simazine, methyl parathion, chlorinated benzenes, chlorpyrifos and metformin (Moore et al. 2013; Wang et al. 2013; Cui et al. 2015). The transformation of organic compounds by *T. angustifolia* has not yet been studied rigorously to date. Thus, there is a clear need to gain further insight into this transformation dynamic. Pentachlorophenol (PCP) is a highly toxic and recalcitrant compound primarily used as biocides in the leather tanning and wood-treatment industries.
Therefore, exposure to PCP in the environment could cause major various health hazards (Karan et al. 2018) as pentachlorophenol is known as a respiratory toxicant with two health effects, non-carcinogenic and carcinogenic. PCP might be an endocrine disruptor and highly toxic to all categories of life (Werheni et al. 2020). Levels of PCP ranging from 25 to 150 mg L\(^{-1}\) were found in slightly alkaline wastewater from wood-treatment facilities (Crosby 1981). Tunisia is one of the countries that signed the European Commission regulation of the 1980s that banned the use of organochlorines (Hassen et al. 2021). Based on this information, the aim of this study was to determine the ability of \(P.\ putida\) HM627618 in PCP removal. Also, the effect of phytoremediation and bioaugmentation process by using \(T.\ angustifolia\), \(L.\ gibba\), and \(P.\ putida\) HM627618, respectively, was examined for PCP removal from contaminated secondary treated wastewater (STWW).

**MATERIALS AND METHODS**

**Wastewater sampling and determination of main physical-chemical characteristics**

Some samples of STWW free of PCP were sampled at scale Charguia I located in a residential and business area of Tunis City, northern of Tunisia. The wastewater sample was stored at 4°C for main physical-chemical characteristics determination and further bioremediation investigations. The cation exchange capacity (CEC) and pH of wastewater were determined according to the standard methods. Total nitrogen was determined using the Kjeldahl method as recommended by Brookes et al. (1985) and total organic carbon content was determined as described by Bouzaian et al. (2009).

**Bioaugmentation essays**

The *Pseudomonas* strain used in this study was isolated from purified plants and identified by its molecular 16S rDNA sequence as *Pseudomonas putida* HM627618 by Mehri et al. (2011). This strain was screening for its plant growth promoting properties (PGPR). This species of *P. putida* HM627618 is usually used in phytoremediation processes showing multifunctional important characteristics, like high pyoverdine production, phosphate solubilization, and indole-3-acetic production (Mehri et al. 2014). The selection of this bacterium in this study was primarily based on its PCP tolerance and removal ability in wastewater, rather than its non-pathogenic characteristic, and considering its plant origin.

The selected *P. putida* HM627618 tolerates and grows at a range of 0–200 mg L\(^{-1}\) of PCP both in liquid mineral salt medium (MSM) and sterile STWW. The growth of the strain *P. putida* HM627618 was achieved in 20 ml of King B broth and incubated at 30 °C for 24 hours. The bacterial culture pellet of the strain *P. putida* HM627618 was recovered after centrifugation at 4,500 rpm for 15 minutes, and washed twice with liquid medium. The composition of the MSM was, in mg L\(^{-1}\): KH\(_2\)PO\(_4\), 800; Na\(_2\)HPO\(_4\), 800; MgSO\(_4\).7H\(_2\)O, 200; CaCl\(_2\).2H\(_2\)O, 10; NH\(_4\)Cl, 500 and 1 ml of trace metal solution comprising in (mg L\(^{-1}\)) FeSO\(_4\).7H\(_2\)O, 5; ZnSO\(_4\).H\(_2\)O, 4; MnSO\(_4\).4H\(_2\)O, 0.2; NiCl\(_2\).6H\(_2\)O, 0.1; H\(_3\)BO\(_3\), 0.1; CoCl\(_2\).6H\(_2\)O, 0.5; ZnCl\(_2\), 0.25; EDTA, 2.5. The PCP solution stock at 10 g L\(^{-1}\) added in all bioremediation experimentations was prepared in methanol and sterilized by autoclaving at 120 °C for 15 minutes. The PCP with >99% purity was provided from Sigma-Aldrich (USA). At the same time, 100 ml of STWW and MSM medium were successively subjected to three autoclaving processes at 120 °C for 30 minutes, cooled at ambient temperature and inoculated with 1 ml of bacterial culture pellet for both treatments STWW and MSM. All analyses were carried out in triplicate.

**Phytoremediation essays**

The phytoremediation process was carried out by using *T. angustifolia* and *L. gibba* as large geographically diffused plants, and the STWW. The STWW was collected from the clarification lagoon at the scale of industrial wastewater treatment of Charguia I, northern suburbs of Tunis, Tunisia. Large numbers of small healthy *T. angustifolia* plants were originally collected from local lagoons and remained in STWW without PCP for six weeks to adapt to their new environment. The selection of plants was based on the height and the number of leaves of each plant in order to achieve a uniformity among groups (Manios et al. 2003). *L. gibba* was placed on filter paper to remove excess water, and transferred to 5 liter plastic tanks for assays. Different treatment of bioaugmentation and phytoremediation was performed 20 days with six combination in contaminated STWW (100 mg L\(^{-1}\)): CN (STWW + PCP), T, L, L + B, T + B, T + B + L. All analyses were carried out in triplicate.
**Table 1** | Physical–chemical secondary wastewater sample parameters

| Parameter        | Unit          | Value         |
|------------------|---------------|---------------|
| pH$_{\text{H}_2\text{O}}$ |               | 7.06 ± 0.13   |
| Conductivity     | µs            | 1.827.07 ± 0.13 |
| COD              | g L$^{-1}$    | 0.4 ± 0.08    |
| Nitrogen         |               | 3.255 ± 0.12  |
| Total organic carbon | g L$^{-1}$ | 1.035 ± 0.015 |
| C/N              |               | 0.30          |
| Chloride         | g Cl$^{-1}$ L$^{-1}$ | 3.125 ± 1.76 |
| Dry matter       | g L$^{-1}$    | 1.46 ± 0.15   |
| MES              | g L$^{-1}$    | 0.605 ± 0.01  |
| DBO$_5$          | g L$^{-1}$    | 0.645         |
| NO$_3$           | mg L$^{-1}$   | 14.6 ± 0.28   |
| SUR              | mg L$^{-1}$   | 64.75 ± 1.77  |

S. WW, secondary wastewater; SUR, surfactant; pH$_{\text{H}_2\text{O}}$, hydrogen potential (1/5); NO$_3$, nitrate; C, total organic carbon; N, nitrogen; DBO$_5$, biochemical oxygen demand; MES, suspended matter; C/N, carbon/nitrogen; COD, chemical oxygen demand.

**Process analysis**

**Bacterial numeration**

The bacterial count was conducted according to the colony-forming units (CFU) method (Kurasvili *et al.* 2016). One milliliter of STWW from each treatment was collected, serially diluted, spread on a *Pseudomonas* sp. agar, incubated for 24 hours at 30 °C, and the number of colonies formed was counted (CFU/ml). All analyses were carried out in triplicate.

**HPLC analysis**

The PCP removal was quantified by high performance liquid chromatography (HPLC). The PCP was extracted by methanol solution by processing 1 ml of different treatments (bioaugmentation, phytoremediation and combined phytoremediation–bioaugmentation process). The wastewater sample had added 1 ml of methanol, and then vortexed vigorously for 5 min and centrifuged at 8,000 rpm for 5 min. The supernatant was filtered through a 0.22 mm cellophane filter and the filtrate was subjected to HPLC analysis by a Perkin Elmer Series YL9100 system instrument as described by Karn *et al.* (2010a). The mobile phase consisted of acetonitrile and phosphoric acid (1% aqueous solution) with a flow rate 1 mL·min$^{-1}$ (PCP retention time was 10 min). The instrument calibration and quantification were performed using the pure reference standard.

![Figure 1](http://iwaponline.com/wst/article-pdf/84/10-11/3091/969045/wst084103091.pdf)

**Figure 1** | Pentachlorophenol determination rates and optical density at 600 nm in mineral salt medium by *P. putida* HM627618 strain over 168 hour at 28 °C.
(1–300 mg L⁻¹). The chromatogram characterizes the concentration presented in the medium. All analyses were carried out in triplicate.

Plant growth: The plant growth was evaluated by leaf and roots biomass dry weight at 80 °C for 24–48 hours and results were expressed by the equations: % relative mass = treated plant dry weight/control plant dry weight × 100. All analyses were carried out in triplicate.

Photosynthetic pigments determination: The leaf samples of *T. angustifolia* and *L. gibba* were cut into 1–2 cm square pieces, frozen, and later analyzed for chlorophylls *a* and *b*. So, 0.2 g of fresh material was mixed in 5 ml of 99.9% acetone, vortexed for 5 min and centrifuged at 5,000 g for 6 min at 4 °C. The absorbance (Abs) was read at 646.8 and 663.2 nm with acetone (99.9%) as the blank (*Lichtenthaler 1987*). The pigments were calculated using the following formulas:

Chlorophyll *a* = \((12.25 × \text{Abs}_{663.2}) - (2.79 × \text{Abs}_{646.8})\)

Chlorophyll *b* = \((21.50 × \text{Abs}_{646.8}) - (5.10 × \text{Abs}_{663.2})\)

Physico-chemical parameters determination: At the beginning and at the end of the various assays carried out, some physical and chemical parameters were determined to evaluate their variations under the PCP effect. These parameters were COD, total organic carbon, chloride, pH, and electrical conductivity (EC) (*Werheni et al. 2021*). All analyses were carried out in triplicate.

Statistical analysis
One-way analysis of SPSS was used to examine the significance among PCP dissipation rates, PCP and physical and chemical analysis. Differences between two parameters were compared in the samples with the Duncan test. All data were presented as mean ± SD (n = 3). Principal component analysis (PCA) was performed in order to evaluate differences among all variables at the end of the experiment (TF) (20 days). The PCA results were shown as a biplot to highlight the interaction between samples and variables. The PCA was performed using the SPSS program package (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.).

RESULTS
Main physical–chemical wastewater characteristics
A summary of the wastewater characteristics is reported in Table 1. The physico-chemical characteristics of STWW were strongly influenced by its main biological properties. For this reason, certain key physical–chemical parameters were analyzed during this study and showed a neutral pH with 7.06 ± 0.12, COD with 0.4 g L⁻¹ ± 0.08, conductivity with 1,827.07 s/cm ± 0.13, and organic carbon content with 1.035 g L⁻¹ ± 0.015. Suspended solid, nitrates, surfactants appeared conform to the Tunisian standard of discharges NT106.02.

Bioaugmentation process
In order to determine the ability of the *P. putida* HM627618 strain to tolerate and use the PCP molecule as a carbon source, different incubations in liquid MSM and STWW medium was artificially contaminated with 100 mg L⁻¹ were carried out (Figure 1). The results of bioaugmentation process were primarily based on the monitoring of *P. putida* HM627618 biomass

| Treatment | pH       | EC (s/cm) | COD (mg L⁻¹) | COT (mg L⁻¹) |
|-----------|----------|-----------|--------------|--------------|
| Wastewater | 9.92 ± 0.035a | 2.067 ± 0.008b | 612 ± 1.33c | 839 ± 0.009d |
| *L. gibba* | 9.84 ± 0.065a | 3.027 ± 0.0007b | 426.66 ± 4.59d | 961 ± 0.005a |
| *T. angustifolia* | 9.835 ± 0.066a | 2.518 ± 0.082c | 820.33 ± 2.148b | 425 ± 0.014b |
| *L. gibba* + *P. putida* HM627618 | 9.843 ± 0.128a | 5.204 ± 0.077b | 810 ± 1.777b | 417 ± 0.003c |
| *T. angustifolia* + *P. putida* HM627618 | 9.55 ± 0.107a | 2.266 ± 0.014c | 850.33 ± 3.370a | 442 ± 0.004d |
| *T. angustifolia* + *L. gibba* + *P. putida* HM627618 | 10.273 ± 0.008a | 3.642 ± 0.020a | 404.66 ± 0.814d | 296.6 ± 0.007c |

COT, total organic carbon; COD, chemical oxygen demand; pH, hydrogen potential; EC, electrical conductivity.
Different lowercase letters indicate significant differences among sampling time in the same treatment assessed by paired t-test (p < 0.05).
and the PCP content in MSM and STWW experiments. The bacterial biomass was determined by monitoring the OD at 600 nm as a function of the incubation time in the various bioaugmentation treatments. Bacterial growth showed a maximum OD of around 1.35 after 144 hours, and at the end of incubation (20 days) only 80 mg L$^{-1}$ of PCP remained, and corresponded to 60% of PCP removal. This PCP removal was initiated from the first day of incubation, which is a good sign of bacterial functioning and with an operative latency period of around 24 hours. In addition, the study of PCP adsorbed to bacterial cells showed around 25 mg L$^{-1}$ after 96 hours of treatment and corresponded to 87.5% of PCP removal (Figure 1). This last result showed that the strain *P. putida* HM627618 could be adsorbed and accumulated a low amount of PCP in its biomass during 96 hours and after this time, this quantity of PCP would be degraded.

**Bioaugmentation and phytoremediation processes**

**Variation of the physico-chemical parameters**

pH and electrical conductivity. We noticed a decrease in pH between days 0 and 5, and from the 6th day an increase in pH was observed until up to the 20th day of incubation for L+B; T+B; L+T+B; L+PCP; T+PCP and NC. This pH averaged 10 ± 0.3 at the end of the incubation period (Table 2). This increase in pH in constructed wetlands at the end of the incubation period could cause some toxicity to macrophytes.

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**Figure 2** | Monitoring of the concentration of pentachlorophenol (a) and variation in the number of bacteria (b) for the various bioaugmentation–phytoremediation treatments in contaminated wastewater (100 mg L$^{-1}$) at 25 °C for 20 days. T: *Typha angustifolia*; L: *Lemna gibba*; PCP: pentachlorophenol; B: *P. putida* HM627618. Different lowercase letters indicate significant differences among sampling time in the same treatment assessed by paired t-test ($p < 0.05$).
Electrical conductivity showed an increase during incubation for L\(\text{+PCP, T+PCP and NC}\) of the order of 3.095, 1.96 and 2.144 and L\(\text{+B, T+B and L+B+T}\) of the order of 3.22, 2.33 and 3.773 s/cm. This increase in EC could be explained by the increase in the concentration of chloride present in STWW.

Total organic carbon. The TOC examination was performed for all PCP experiments. TOC is one of the most important comprehensive parameters when evaluating the organic pollution of wastewater and the general condition of STWW. Table 2 shows the TOC registered for the different treatments conducted under controlled conditions during the 20 days incubation. TOC at the beginning of the incubation experiments was around 900 mg L\(^{-1}\). After 20 days, especially for L\(\text{+T+B+PCP: L+B+PCP: NC and L+PCP}\), TOC showed a significant decrease that could be below 455 mg L\(^{-1}\). This result could be explained by phytoremediation–bioaugmentation operative and process functioning, which uses available carbon in the STWW for plant and bacteria growth and biomass production.

Chemical oxygen demand (COD). The increase in COD in STWW at the end of the experiment affected the survival and growth of *T. angustifolia* and *L. gibba* plants. Furthermore, at the end of incubation, COD increased more than twice compared to the beginning of incubation. The highest COD was detected in the treatment L\(\text{+T+PCP+P. putida}\)}
HM627618 with a value 850 mg L\(^{-1}\) at TF. Compared with the phytoremediation–bioaugmentation processes, the phytoremediation treatment alone showed a final low COD. The COD could promote the growth of plants and offer better biological activity. In the ex-situ process, the increase in COD could cause poisoning and early death of *T. angustifolia* macrophytes and *L. gibba*, which could be evaluated by chlorophylls a and b measurement.

**Evolution of PCP**

During all bioremediation experiments, the PCP content showed a net decrease according to the time of incubation (Figure 2(a)). The PCP removal by *Typha* appeared to be more effective than the one achieved by *L. gibba*, with a residual PCP 22.2 mg L\(^{-1}\) (77.8% removal) and 45.068 mg L\(^{-1}\) (54.63% degradation), respectively. Moreover, the PCP removal in the negative control treatment (STWW + PCP) was low, with 73.03 mg L\(^{-1}\) (26.97% removal). Conversely, the combined treatment of *T. angustifolia* and *P. putida* HM627618 strain as the promoting growth (PGPR) strain showed a lower PCP removal compared with the one achieved by *T. angustifolia* alone, with a residual PCP of 12.62 mg L\(^{-1}\) (77.8% degradation). *P. putida* HM627618 strain is considered as a good PGPR *Pseudomonas* strain and could play an important role for promoting growth of plants by increasing its adaptation, acclimatization and tolerance to stress.

*Figure 4* | Changes in chlorophylls a and b for *Typha angustifolia* (T, T + B and T + L + B) and *Lemna gibba* (L, L + B L + PCP and L + T + B) macrophyte in the different phytoremediation processes. L : *Lemna*; T : *Typha*; T + B : *Typha* + *P. putida* HM627618 ; T + L + B : *Typha* + *Lemna* + PsTp139.
Evolution of the number of strain *P. putida* HM627618

Figure 2(b) shows the number of *P. putida* HM627618 strain found on the agar medium for the different STWW treatments, at T0 (beginning of treatment) and after 20 days (end of treatment). The results showed a gradual increase of the number of bacteria, reaching a maximum of $1.1 \times 10^6$ CFU·mL$^{-1}$ for the L$^+$$B$ treatment; and from the 11th day, the number of bacteria started to decrease gradually until 20th day, with a value $8 \times 10^4$ CFU·mL$^{-1}$. This decrease in the number of bacteria could be explained by the tolerance of bacteria to PCP in STWW, and the lessening of organic and mineral compounds required for microbial growth.

Variation of chloride content. Chloride is generally harmless at high concentrations for natural life. For the 20 days of the phytoremediation and bioaugmentation processes, the evolution of chloride content is shown in Figure 3. The result showed a net increase in chloride from the start to the 8th day of incubation for all treatments. From the 9 or 10th days, the chloride showed a clear decrease until the end of the bioremediation experiments. This result could be explained and related to the fate...
of PCP in the medium. The decrease of chloride corresponded to some complex phenomena, i.e., PCP mineralization and dechlorination, root biosorption and biomass adsorption that might be proportional to the PCP transformation, disappearance and/or removal.

*Photosynthetic pigments variation.* In order to verify the adaptability of plants to various compounds and environments, the chlorophyll content is usually assessed as a physiological indicator element. In addition, the various nutrients and toxic compounds in wastewater could be incorporated and assimilated by the macrophytes and that affected thoroughly the chlorophyll synthesis. The amounts of chlorophylls a and b, found for *T. angustifolia* treatments added with PCP, were high with 25 and 32 μg per mg of fresh biomass, respectively (Figure 4). The same treatments carried out by *L. gibba* showed values lower than 25 μg per mg of fresh biomass. These last results established that *T. angustifolia* has a high capacity of tolerance to toxic compounds compared with *L. gibba* and it could be a more suitable candidate for toxic wastewater treatment, like industrial. Also, this last finding could suggest relevant information for further phytoremediation studies and their practical implication.

*Principal component analysis (PCA).* In order to summarize the changes observed in the phytoremediation–bioaugmentation treatment of PCP-contaminated secondary wastewater during the incubation period, the different treatments were illustrated by the principal component analysis (PCA) (Figure 5(a)). PCA graphically distinguishes different wastewater samples (L + B; T + B; L + T + ; L + PCP; T + PCP and NC) based on the sampling time (T0: start of the experiment and TF: end of the experiment). The two main components accounted for 70.72% of the total variance. The treatments: T + B and L + B were positively correlated with chloride and bacteria number and COD parameter variation.

**DISCUSSION**

According to the results obtained in this research, the selected species of the strain *P. putida* HM627618 was able to tolerate and eliminate the toxic and recalcitrant PCP in secondary wastewater at 100 mg L⁻¹. After 168 hours of incubation, strain *P. putida* HM627618 showed 90% PCP removal initially when added in the treatment. It is well known that the *Pseudomonas* genera show significant physiological and metabolic adaptability, authorizing and approving colonization of numerous terrestrial and aquatic environments (Joshi et al. 2015), this important characteristic strengthens and supports their increasing use and application alone or as consortium tools in biotechnological bioremediation processes. Numerous studies have shown and confirmed the ability of *Pseudomonas* genera to degrade some xenobiotic and recalcitrant compounds such as PCP, for example *P. putida* SKG-1 MTCC (Garg et al. 2013); *P. putida* CL7 (Karn et al. 2010a, 2010b); *P. aeruginosa* PCP2 (Sharma & Thakur 2008); *P. mendocina* NSYSU (Kao et al. 2004); *P. veronii* PH-05 (Nam et al. 2003); *P. aeruginosa* (Premalatha & Rajakumar 1994); *Pseudomonas* sp. strain RA2 (Radehaus & Schmidt 1992) and *Pseudomonas fluorescens* (Werheni et al. 2015).

Our results are in agreement with other studies, which reported that these kinds of bacteria are effective microorganisms in remediation of pesticide-polluted sites, and could be highly active micro-degraders (Doolotkeldieva et al. 2018). Moreover, Radehaus & Schmidt (1992) indicated that PCP addition to the culture medium led to a prolonged and extended initial growth phase of *Pseudomonas*; this conduct and behavior could be explained by the period of *Pseudomonas* acclimatization and adaptation to the contaminated and hostile environments.

In addition, our results showed a reduced number of days needed for 160 mg L⁻¹ of PCP degradation by the strain *P. putida* HM627618 compared with the number of days obtained in the case of another species of *Pseudomonas putida* and for the same PCP concentration (Radehaus & Schmidt 1992).

Also, our study showed that a low quantity of PCP was adsorbed and bioaccumulated in the microbial biomass of the strain *P. putida* HM627618. The study of Hassen et al. (2018) dealing with PCP bioremediation reported that *Pseudomonas putida* addition to the growth medium promoted the bioaccumulation, adsorption and biosorption process in the bacterial biomass.

Conversely, the bioaugmentation process conducted in this study by using two macrophytes, fixed (*T. angustifolia*) and floating (*L. gibba*) macrophytes for PCP removal from contaminated secondary wastewater. The results found indicated that *T. angustifolia* played a very important role in removing more PCP than *L. gibba*. In the presence of these two macrophytes, 100 mg L⁻¹ PCP were removed from secondary wastewater after 9 days of treatment. In a similar study, Ferro et al. (1999) confirmed that the PCP degradation was favored by the presence of plants. Also, Ennabili et al. (2019) showed that
L. gibba tolerated highly polluted sites, because of their aptitude to eliminate carbon and assimilate nitrogen and phosphorus. L. gibba is one of the many macrophytes experimentally used in Morocco for urban wastewater treatment. However, little information was available in the literature regarding the effectiveness of L. gibba for chlorophenol remediation. Moreover, the study of Werheni et al. (2021) revealed that T. angustifolia showed a capability to tolerate and eliminate a PCP in wastewater at 100 mg L\(^{-1}\). Also, results of the present work showed that PCP removal was proportional to chloride release in STWW, and this release was influenced by the growth plant degree. The chloride content augmented according to the reduction of PCP in wastewater. Figure 5(b) shows the response surface plot indicating an important interaction effect between residual PCP (mg L\(^{-1}\)), chloride content and time during PCP phytoremediation and bioaugmentation. The quantity of PCP utilized and the release of chloride in the medium registered during the operational time of bioremediation could be assigned mainly to mineralization of PCP (Karan et al. 2018).

In addition, the variation of the pH in the wastewater was very important to its vital and crucial role regarding the microbial growth and the PCP mineralization. This pH would be optimal between 6.5 and 8 (Hechmi et al. 2013). Finally, the growth and PCP removal by emergent wetland plants might be largely influenced by the average bio-physico-chemical characteristics dominating in the site (Hechmi et al. 2013). The phytoremediation process is largely considered as an easy, cost-effective, and eco-friendly method that has emerged as an alternative technology using aquatic plants or macrophytes for removal of contaminants from water and soils (Werheni et al. 2021). Additionally, this process is technologically feasible and implies low operating costs, least possible sludge generation and competitive performance (Khataee et al. 2012; Sasmaz et al. 2015; Verma & Suthar 2015).

Several experiments have demonstrated the power of plants and trees to degrade recalcitrant or poorly degraded environmental contaminants by plant enzymatic systems and rhizospheric microorganisms.

**CONCLUSION**

The selected P. putida HM627618 strain appeared capable of eliminating and accumulating PCP at a concentration of 100 mg L\(^{-1}\). Results obtained by HPLC analysis showed that PCP mineralization takes place firstly, following the phenomenon of adsorption of PCP on the bacterial cells, secondly succeeding to PCP intracellular accumulation. The evaluation of the phytoremediation (T. angustifolia and L. gibba) and phytoremediation-bioaugmentation (P. putida HM627618) efficiency was followed and performed at a PCP concentration of 100 mg L\(^{-1}\). The T. angustifolia plant has been shown to be more resistant to stressful environmental conditions via and thanks to these roots accumulating PCP according to the known phenomenon of rhizofixation. The use of P. putida HM627618 bacteria can ameliorate PCP removal in STWW in the lagoon system. Thus, this phytoremediation technique, which uses aquatic plants, offers potential benefits for the remediation of the contaminated environment by improving biological procedures.

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**DATA AVAILABILITY STATEMENT**

All relevant data are included in the paper or its Supplementary Information.

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