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Assessment of Ornamental Plant *Ruellia simplex* and Bio-Adsorbent for Removal of Amoxicillin and Ciprofloxacin – A Preliminary Test for Constructed Wetlands

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ABSTRACT

One of the procedures that helps remove pharmaceuticals (Phs) from wastewater in Constructed Wetlands (CWs) is phytoremediation. Using *Ruellia simplex*, the main emphasis of this study is on the function of plants and substrates in the removal mechanism independently from aqueous solution. Even in an aqueous solution containing high concentrations of antibiotics, the plant could thrive, and lighting further promoted growth. For the removal of amoxicillin (AMX) and ciprofloxacin (CIP) at a concentration of 25 mg/L, six reactors are used: hydroponic planted reactors, sand rectors, chicken manure – wood shavings (CM-WS) rectors, rhizobacterial-inoculated unplanted reactors, unplanted control reactors, and unplanted dark control reactors to study the roles of phytoremediation, adsorption, biodegradation, photodegradation and hydrolysis process in reduction of AMX and CIP. Additionally, the plant's weight is determined for each sample collected and compared to the initial weight of the experiment to monitor plant growth. The possible fates of AMX and CIP were explored using hydroponic tests, whereby 43%, 26% and 19%, respectively, of the total removal of AMX had been accounted for by plant uptake, adsorption by CM-WS and biodegradation after 21 days of exposure, also these process contributed well in CIP removal, where the proportions were 39%, 32% and 18%, respectively of the total CIP reduction at the same intervals.

Keywords: *Ruellia simplex***,** amoxicillin, ciprofloxacin, hydroponic, chicken manure, wood shavings.

INTRODUCTION

Antibiotics are often used to treat and prevent diseases in both people and animals. Although antibiotics are helpful for many organisms, a large quantity of these medications end up in the environment when humans and animals urinate and defecate. Consequently, antibiotic-resistant genes and microorganisms have emerged, persisted, and proliferated in the environment due to the overuse and abuse of antibiotics. The recent finding of antibiotics in soil and water poses a particular threat to ecosystems and human health. Consequently, we must act immediately to address the problem of antibiotic contamination (Zhi et al., 2019). A semi-synthetic β-lactam antibiotic, amoxicillin (AMX) is frequently used to treat a variety of diseases in human medicine. It serves as a development promoter in veterinary medicine as well. Since AMX has a low rate of metabolism when it leaves the body, environmental samples containing it are more likely to contain it than metabolites. It has been found in environmental samples, such as surface water and secondary treated effluents, at quantities in the μg/L range. AMX concentrations in antibiotic production effluents occasionally approach the mg/L mark (Das et al., 2018). Ciprofloxacin, also known as C17H18FN3, fluoroquinolone group, or CIP, is one of the most often used antibiotics to treat various bacteria that affect the human body. Gram-positive and gram-negative bacteria may both be effectively treated with ciprofloxacin. Numerous investigations have shown the in biodegradability of CIP and the release of microorganisms resistant to ciprofloxacin from residential wastewater treatment facilities (Lin et al., 2017).

Plant, substrate, and microbial growth have been shown to have a significant impact on the treatment of polluted water in CWs. The relationship and interaction with plants and the community of bacteria also affects how well phytoremediation removes contaminants (Alsalihy et al., 2024). Experiments in hydroponics have been used to show how various medications are absorbed by plants. Plant species are exposed to known pharmacological concentrations for a given amount of time in these kinds of research. There are a number of plant types that may absorb medications from soil and water. It's interesting to note that lettuce (*Lactuca sativa* L.) and carrots (*Daucus corota* L.), two edible plants, have been shown to absorb antibiotics from irrigation water (Mzukisi et al., 2018).

Ruellia is a genus of flowering plants in the *Acanthaceae* family that is sometimes referred to as wild petunias or ruellias. There are over 2500 species and 250 genera in it. Some of them are epiphytes, but the majority are shrubs or twining vines. In temperate locations, only a few species are found. They are dispersed across Pakistan, Central America, Brazil, Africa, Indonesia, and Malaysia (Ahmad et al., 2015). The *Ruellia simplex or Ruellia brittoniana* or *Mexican petunia* is a native of Mexico; however, it has evaded cultivation and settled in disturbed areas in the southeastern United States (Ardous et al., 2020). It is also considered a perennial herb that can grow up to one meter in height. Green or purple stems. The leaves are lance-shaped, dark green, and opposite, measuring approximately 15–30 cm in length and 1–2 cm in width. The veins are conspicuous below the leaves, and the margins are either smooth or undulating. The flowers are pedunculate, trumpet-shaped, and typically isolated or grown in clusters at the ends of the stems. They are 4–8 cm in diameter and are typically purple, although white and pink varieties are also present. Seeds are dispersed over extensive distances as a result of the explosive dehiscence of capsules. They are adaptable plants that are highly capable of withstanding both drought and inundation. It is capable of flourishing in a variety of environments, from full sunlight to partial shade, and it benefits from consistent watering during the summer (Langeland et al., 2008).

A large number of CWs are placed on natural substrates like soil, gravel, sand, or substrates derived from silica. Bio-adsorbent materials like activated carbon, when added to CWs containing sand, increased antibiotic removal from 18% to >80%. This might be because of the increased adsorption capacity and the larger number of microorganisms that break down antibiotics (Cui et al., 2023). Using sand, an adsorbent substance, to treat wastewater has many positive effects on both the economy and the environment. The potential of sand as a solvent remover has been the subject of much study. Consequently, it is preferable to seek out inexpensive, efficient adsorbents like sand derivatives in order to reduce the high price and complexity of regeneration (Bello et al., 2013). In order to improve pollutant removal in CWs while treating wastewaters, some earlier researchers used additional novel carbon sources as papers, Apricot pit, shell walnuts, food manufacturing waste, wheat waste and others plant waste (Wang et al., 2019). Biochar, which is produced from feedstocks such as cattle excrement, poultry manure, rice straw, porcine grass, and maize stover, is effective in the removal of pollutants from effluent (Jagadeesh and Sundaram, 2023). The utilization of animal refuse to treat pollutants, including those from cattle, poultry, and pigs, offers numerous advantages. In the last decade, there has been a substantial increase in the yield of poultry manure. The environmental contamination caused by poultry manure has become a significant social problem, and it has the potential to generate health risks for humans and animals hazards, aromas, and the leakage of nitrates and other contaminants into groundwater. Nevertheless, poultry manure is an important resource as a soil fertilizer, as it can provide a significant amount of macro- and micro-nutrients for crop growth. Consequently, it is imperative to develop effective technologies for recycling the waste and reducing its negative impact on the environment (Gao et al., 2010). The biomass of poultry manure is increasing annually as the number of fowl increases. Subsequently, producers frequently apply poultry manure to agricultural land as a disposal method. Nevertheless, the emission of methane, ammonia, or other organic compounds from composted CM poses a substantial hazard to water and air pollutions. The majority of prior research has focused on the utilization of CM for the production of biodiesel and energy recovery. Few studies have explored the potential of poultry manure as an adsorbent for the capture of CO_2 and the adsorption of pollutants (Quang et al., 2019). Using unprocessed manure as an organic fertilizer without prior treatment has adverse environmental consequences. This practice functions as a vector for the propagation of pathogens, attracting flies and vermin, and contributing to pollution problems. The addition of compost to soil improves the organic matter content, soil structure, and agricultural productivity using the presence of plant growth-promoting organisms and the additional nutrients in the compost (Manogaran et al., 2022). Anaerobic digestion is a promising technology for using organic waste; however, it is necessary to dilute waste with a high nitrogen content, such as manure, to reduce the ammonia inhibition effect. This results in the production of a substantial quantity of effluent that is difficult to utilize (Shapovalov et al., 2020). A chemical process known as photolysis occurs when a substance is either directly or indirectly broken down by light. Light chromophores absorb light, which causes direct photolysis; reactive species, created by photosensitization, emit light, which causes indirect photolysis. These actions have a major impact on water ecosystems (Eduarda et al., 2023).

The removal of Phs by phytoremediation is the sustainable and advantageous method that is the subject of this research. A study on the use of a plant that is suitable for the climate in Iraq and is evergreen, fast-growing and able to grow well in the presence of Phs contaminants in the wastewater is limited. The use of animal waste in CWs is very limited. There is a study that dealt with the use of cow manure (Singh and Chakraborty, 2020) and a study that used goat manure, wood shavings and soil (Sheoran, 2017) to remove pollutants from wastewater, but there were no pharmaceutical pollutants. CM is used in the removal of many pollutants such as naphthalene (Liu et al., 2021), phenol and 2,4-dinitrophenol (Quang et al., 2019), mercury (Klasson et al., 2009) and dimethyl sulfide (Nguyen and Lee, 2015). Utilization of CM to produce biochar proved high efficiency for the removal of some pharmaceuticals like levofloxacin hydrochloride (Ji et al., 2022) and tetracycline and rhodamine (Mercurio et al., 2023). Several studies have proven the efficiency of natural and modified agricultural waste in removing antibiotics (Eniola et al., 2019; Hoslett et al., 2021; and Nordin et al., 2023). The mixture of CM-WS to remediate heavy metals was studied by (Lima et al., 2015). Furthermore, there is not enough of research that adequately discusses the roles played by every process in removal, including hydrolysis, photodegradation, biodegradation, adsorption, and phytoremediation. Additionally, there is a lack of knowledge on the behaviour and processes of Phs in CWs, particularly the relationships between microbes, plants, and substrate in the process of Phs degradation. This study aims to fill up some of the prior information gaps. Therefore, the goals of the review are:

1. To monitor the potential fate of AMX and CIP in CWs through hydroponic experiments, as well as assess the plant's efficacy in Phs removal by using *Ruellia simplex*;
2. To study the use of animal waste (chicken manure) with agriculture waste (wood shavings) as alternative materials in a substrate within the layers of CWs with high efficiency, thereby reducing costs and high hydraulic conductivity;
3. To determine the role of other processes in the removal process, including biodegradation, adsorption, photolysis, and hydrolysis, in addition to plant uptake.

MATERIALS AND METHODS

Chemicals and reagents

The antibiotic ciprofloxacin (CIP) was of 98% purity, whereas amoxicillin trihydrate (AMX) was of >95% purity. All of these products have been supplied by Sigma-Aldrich (Germany). Sigma-Aldrich (Germany) provided all of the reagents necessary for the antibiotic quantification, all of which were of HPLC grade.

The reactor experiments

For each investigated substance, the studies were carried out in vitro in 500 mL cylindrical bottles at room temperature (20 ±5 ºC) from the end of January to February in Baghdad University/Iraq, as shown in (Fig. 1 and Fig. 2). This was accomplished by establishing a set of eighteen cylindrical bottles that included hydroponic planted reactors (a), sand rectors (b), chicken manure - wood shavings (CM-WS) rectors (c), unplanted reactors infected with rhizobacteria (d), unplanted control reactors (e), and unplanted dark control reactors (f).

Reactor A used hydroponics to grow a single plant that was left exposed to natural light in order to evaluate the impact of plant absorption and translocation. Reactor B received the addition of 100 g of sand to validate the adsorption function of sand. Reactor C received an additional 100 g of chicken manure - wood shavings (CM-WS) to further demonstrate the function of adsorption. CM-WS was collected from a local chicken farm in Baghdad. In poultry farms, wood shavings are combined with CM to provide an environmentally favorable surface and an optimal floor covering for the fowl. The presence of a high concentration of microorganisms in CM poses a significant risk to crops. Consequently, it is imperative to conduct a substantial fermentation of CM to eliminate parasites, parasite ova, and certain infectivity bacteria during the high-temperature maturation process. The traditional method of fermenting CM on a small scale, such as fermenting the refuse of small poultry farms to utilize it as fertilizer for cultivated plants, is employed. The subsequent procedures are implemented: spread a plastic bag with a diameter of 5–6 meters on the ground, secure one

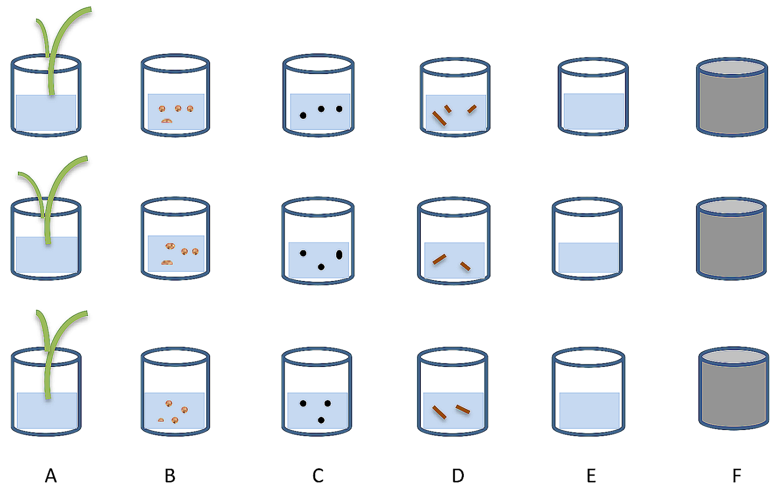

Figure 1. Sketch diagram of the hydroponic experiments: (a) hydroponic planted reactors, (b) sand rectors, (c) chicken manure - wood shavings rectors, (d) rhizobacterial-inoculated unplanted reactors, (e) unplanted control reactors, and (f) unplanted dark control reactors

Figure 2. Hydroponic experiments setup

end, and fill it with poultry manure in a quantity between 75 and 100 kg. Then, water is introduced to the manure until it is fully saturated. Ultimately, the sack is sealed for 2–3 months, contingent upon the climate of the surrounding environment. During this period, the manure is unsealed and rotated over, and water is added as required to ensure that it is entirely decomposed. The CM-WS after fermentation is shown in (Fig. 3).

Reactor D was immediately injected with certain amount of rhizobacteria by shaking the plant roots in the solution for a few seconds in order to evaluate the effect of biodegradation. In order to reduce the possible photolysis impact of target compounds, two control reactors (containing the pollutant alone) were added. The first, (E), was left exposed to natural light, while the second, (F), was entirely covered with aluminum foil.

The removal of the antibiotic through hydrolysis, photolysis, and plant uptake in both illuminated and dark environments was investigated by (Habaki et al., 2023). The hydrolysis reaction was assumed to be reversible and occur in all cases, while the photolysis reaction was assumed to be irreversible and only occur under illumination conditions. This leads us to the conclusion that hydrolysis can be investigated in the dark state, as it is the only dominant process in removal, despite its presence in all other reactors. Therefore, the hydrolysis process will be studied separately by measuring the decrease in the concentration of pollutants in reactorc F only.

The concentration of antibiotic in solution was 25 mg/L. This concentration was chosen to be consistent with what was dependent in (Olama et al., 2018; Verma and Haritash, 2019; and Zhou et al. 2024). 300 mL of AMX and CIP diluted solution were injected into the reactors. Throughout the exposure periods, the water loss from the reactors (from evaporation and/or transpiration by the plants) was continually measured and replenished with deionized water to restore the original volume. 3, 7, 12, and 21 days were the retention intervals used in the trials. Following the reactors' harvest at the conclusion of each retention period, plants and effluent were gathered and examined.

Sampling procedure

During hydroponic test, analyses were performed by high performance liquid chromatography (HPLC) model (SYKAM- German) at the Department of Environment and Water, Ministry of Science and Technology, Iraq. It was equipped with a 100 µl injection valve, a UV/Vis detector set to 278 nm, and a C18 reverse phase analytical column (25 cm × 4.6 mm and 3.5 µm). The mobile phase was progressively a mixture of acetonitrile, methanol, and 0.025 M phosphoric acid, which was adjusted to pH 3 with triethanolamine. The flow rate was 1.0 ml/min. Preparation of standard compounds was taken 0.1 g of each standard compound (CIP) separately and placed in a 250 ml volumetric flask and the appropriate solvent was added to it. Where the initial concentration became (400 ppm) using the dilution law, the concentrations that were injected into the device were prepared. Mobile phase consisted of 5.0% CAN-14.25% CH_3OH-80.75% buffer solution (0.2 M NaOH+ 0.2 M KH_2PO_4), pH = 8.5, flow rate 1.0 mL/min and wavelength 230 nm for AMX.

Equation 1 was used to ascertain the removal efficiencies (R) of the target compounds at the conclusion of each retention interval (3, 7, 12, and 21 days) by comparing their initial concentration (25 mg/L) with their measured concentrations. Based on the variations in the removal efficiencies determined in the reactors after each

Figure 3. The mixture of CM-WS

retention interval, the magnitude of each potentially-involved removal mechanism, that include plant uptake, adsorption, biodegradation, photolysis, hydrolysis, and residual, was approximately estimated.

The initial and final concentrations in the synthetic effluent were used to calculate the removal efficiency of the target compounds in accordance with Eq. 1:

$$R\% = \frac{Ci - Ct}{Ci} \times 100 \quad (1)$$

where: R is the removal efficiency (%); Ci and Ct are the pharmaceutical concentration at initial and through time intervals (mg/L).

The plants get removed from the solution and the deceased detritus is eliminated if it is discovered. Plants are weighed five times: at the commencement of the experiment, after three days, seven days, twelve days, and at the end of the experiment. The correct selection of parameters is corroborated by the observation that reactor A exhibited the development of healthy phytomass under these conditions (Brunhoferova et al., 2021).

RESULTS AND DISCUSSION

The reduction of AMX & CIP concentration by *Ruellia simplex*, sand and chicken manure – wood shavings (CM-WS)

Visually, exposure of *Ruellia simplex* to AMX & CIP did not render adverse visible impacts to plant growth, (Fig. 4 and Fig. 5) shows the removal efficiencies of AMX and CIP from the aqueous solution after 4 retention intervals (3, 7, 12 and 21 days). The concentrations of AMX & CIP in aqueous solution decreased with time in all reactors. Strong positive correlations of 0.88, 0.92, 0.88, 0.92, 0.84 and 0.79 were observed between the retention time and the removal rates of AMX for reactors A, B, C, D, E and F, respectively. Moreover the correlations coefficient of CIP in A, B, C, D, E and F were 0.92, 0.87, 0.92, 0.94, 0.86 and 0.78.

It has been noted that AMX concentration decreased to 38.5%, 22.3%, and 19.3% of the initial concentration after only 72 hr of exposure in reactors A, C, D respectively. Approximately 80.6% of AMX concentration was removed from the aqueous

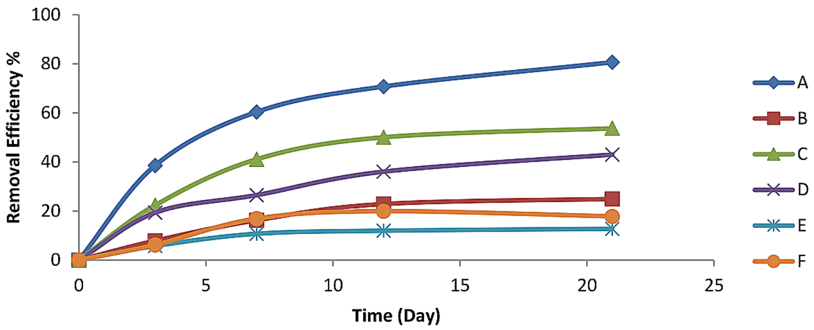

Figure 4. Removal of AMX from aqueous solution in the rectors, (a) hydroponic planted reactors, (b) sand rectors, (c) chicken manure - wood shavings rectors, (d) rhizobacterial-inoculated unplanted reactors, (e) unplanted control reactors and (f) unplanted dark control reactors.

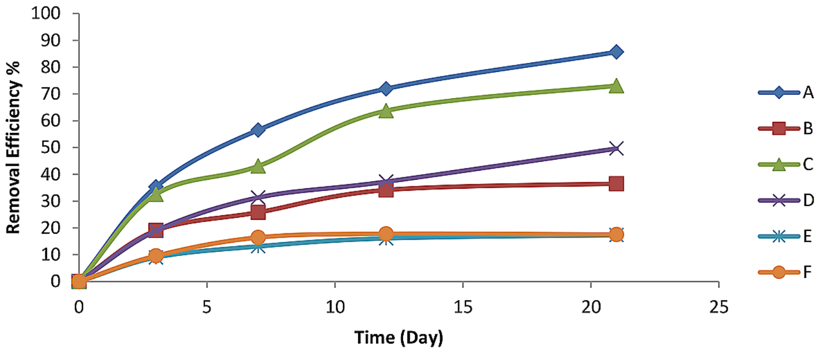

Figure 5. Removal of CIP from aqueous solution in the rectors, (a) hydroponic planted reactors, (b) sand rectors, (c) chicken manure - wood shavings rectors, (d) rhizobacterial-inoculated unplanted reactors, (e) unplanted control reactors and (f) unplanted dark control reactors.

solution at reactor A after a 21-day retention interval. In addition 53.6% and 43% removals in reactors C and D (Fig. 4). In contrast, only limited removals 24.8%, 12.7 and 17.8, were achieved in reactors B, E and F, respectively, for the same interval.

Moreover, the removal pattern of CIP was almost near to that observed in AMX reactors, as the removal rates in the reactors A, C and D were also higher than others reactor, reaching up to 85.6%, 73% and 49.6% after a 21-day retention interval (Fig. 5). Growth development of reactor A at the beginning, 3, 7, 12, and 21 days shows in (Fig. 6). The results showed an increase in plant weight from the beginning of the experiment to its end, where the increase in plant weight was approximately (8–11) gm from the beginning of the experiment. This represents an increase in weight by a percentage 24%, 16% and 15% for A1, A2 and A3 reactors, respectively.

Estimating the percentages of removal mechanisms

The high removal rates achieved in reactors A, C and D (Fig. 4 and Fig. 5) indicated the effective role of phytoremediation by plant, adsorption by CM-WS and biodegradation in the reduction AMX and CIP. The removal process is classified into phytoremediation, adsorption, biodegradation, photodegradation and hydrolysis which was evident in control reactors, albeit marginally, cannot be overlooked. The role of each removal process was assessed based on comparing the decrease of AMX or CIP concentrations in the aqueous solutions during the successive retention intervals; then results were normalized as depicted in (Fig. 7 and Fig. 8).

The effect of plant uptake

The rate of removal in the planted reactors (A) and the control reactors (E), which were plant-free, were compared to estimate the role of plant intake in AMX and CIP. As depicted in (Fig. 7 and Fig. 8), plant uptake had the highest removal contribution for both AMX and CIP throughout the experiment intervals.

Plant uptake constituted 21% of the total removal of AMX after 72 hr. retention interval only, and linearly increased to 43% after 21days. The

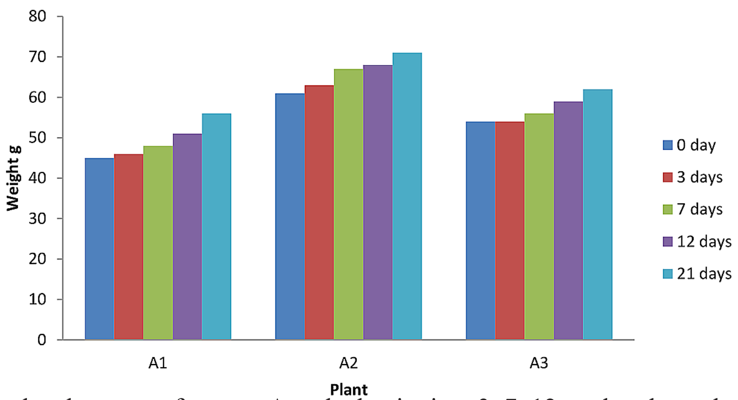

Figure 6. Growth development of reactor A at the beginning, 3, 7, 12, and at the end of the experiment

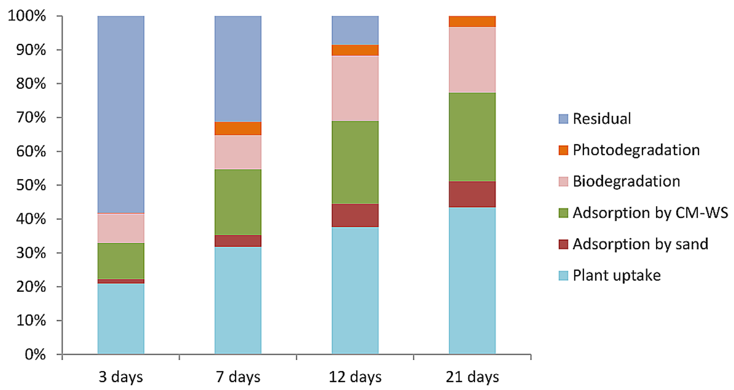

Figure 7. The removal percent of AMX classified as plant uptake, adsorption, biodegradation, photodegradation and residual in the aqueous solution at 4 intervals

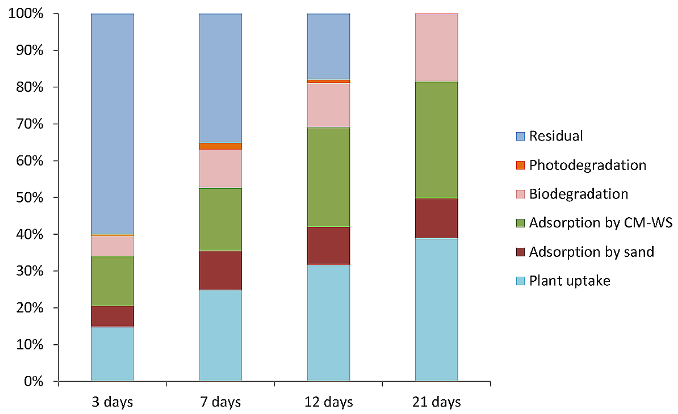

Figure 8. The removal percent of CIP as classified plant uptake, adsorption, biodegradation, photodegradation and residual in the aqueous solution at 4 intervals

AMX-uptake rates observed in this study are consistent with those reported by Pedrosa et al. (2020), where investigated 640 leaves of L. minor (about 1.87 g of plant fresh weight) and succeeded to eliminate approximately 50% of AMX from 100 ml of medium for growth after 14 days.

Furthermore, plant uptake constituted 15% of the total removal of CIP after 72 hr. retention interval, and linearly increased to 39% after 21 days. Adesanya et al. (2021) discovered during the 21-day growth period that the percentage plant uptake values of the antibiotic were much higher for cattail (34% CIP) than for switchgrass (10% CIP). The selection of the optimal plant variety after a battery of experiments on several evergreen plant varieties that withstand variations in summer and winter temperatures accounts for the observable difference in efficiency. CWs, in the presence of plant species, have proven highly efficient in removing AMX and CIP, as mentioned in (Alsalihy et al., 2024).

The effect of adsorption on AMX and CIP removal

In this study, the rate of the adsorption process in both sand, CM-WS were verified to determine the benefit obtained in the removal process when CM-WS mixture is added to the sand layers in CWs. Adsorption was evaluated based on the decline in pollutant concentrations in reactor B and reactor C then compared with the results of control reactor E.

The adsorption percent of AMX and CIP on sand was found to be negligible as it not exceeded 8% and 11%, respectively of the total removal, while the removal percent of CM-WS in AMX was up to 26 % and the adsorption efficiency of CIP on cm-ws was higher from AMX reach to 32%. The removal efficacy of this mixture is attributed to its high content of organic matter, which makes it a suitable absorbent. Adeyemo et al. (2019) mentioned that poultry manure has a higher proportion of organic carbon than other organic sources. The majority of CM is composed of carbon, followed by nitrogen, phosphorus, oxygen, and hydrogen. The carbon content is the most essential variable in the removal mechanism (Quang et al., 2019). Additionally, the surface area of the CM reached 21.3 $m^2 \cdot g^{-1}$ and contains organic functional groups as reported by (Batool et al., 2017).

The hydrophilic nature of the target antibiotics accounted for their low removal efficiency by sand filtration, as explained by Rizzo et al. (2015). The process of target molecule adsorption on suspended substances and their concurrent trapping in the sand bed may have been the cause of the parallel removal of Phs that resulted from the removal of suspended solids by sand filtration. Occasionally, the target pharmaceuticals were released/desorbed from the sand bed due to a higher concentration in the effluent of the sand filtration unit than in the influent. Clean sand and coated sand were both capable of absorbing CIP, as demonstrated by Chen et al. (2013); however, Their capabilities varied considerably. Furthermore, the Langmuir model determined that the affinity of binding locations on the coated sand was 6 times greater than that observed on the clean sand. Furthermore, the sorption of CIP onto the coated sand was significantly higher than that on the clean sand. The minuscule amount of CIP that purified sand absorbed is likely due to columbic interaction, despite the fact that it is predominantly constituted of SiO_2. It has been observed that the removal rate of CIP by adsorption is higher than that of AMX, and this agrees with Cela-dablanca et al. (2024) who investigated the adsorption of CIP and AMX on bio-adsorbents and agricultural soils and discovered that CIP continuously showed better adsorption than AMX, especially at increasing concentrations. Compared to soils, bio adsorbents especially pine bark and oak ash showed greater adsorption capabilities.

The effect of biodegradation

The rates of degradation of reactors (D) were compared to those of control reactors (E) in order to assess the role of biodegradation. Accordingly, the increase in removal rates can be attributed to biodegradation, which represented (9–19)% of the total removal of AMX and the removing of CIP approximately (6–18)% throughout the experiment. Regarding AMX, biodegradation contributed at rates close to those achieved for CIP. The results indicate a promising removal rate, as there are several studies that have proven the efficiency of biological degradation in removing AMX and CIP. Yang et al. (2020) investigated the decomposition of AMX by antibiotic-degrading bacterial strains, SF1 (*Pseudmonas* sp.) and A12 (*Pseudmonas* sp.). The strain SF1 exhibited a degradation rate of 89.1% after 8 days of incubation, while strain A12 exhibited a degradation rate of 81.4% after 8 days of incubation. The combined degradation rate of strains SF1 and A12 was 93.4% after 8 days of incubation. An efficient CIP-degrading bacterium, *Paraclostridium* sp. (strain S2), was immobilised in a study conducted by Fang et al. (2022) to improve the removal of CIP (1000–10,000 μg/L) in pharmaceutical wastewater. In the bioaugmented bioreactor with immobilised strain S2, the acute toxicity of the effluent was significantly reduced by 81.1 % when compared to that of the control bioreactor. Additionally, the bioaugmented bioreactor with immobilised strain S2 increased CIP removal by 18.7 % compared to the control bioreactor for high CIP concentrations (i.e., 10,000 μg/L). According to Zhou et al. (2024), biodegradation by P. bifermentans became the main route for higher concentrations, with biodegradation rates ranging from 34.4% to 50.7% at starting CIP concentrations of 5 mg/L to 20 mg/L.

Role of photolysis and hydrolysis

The photolysis percentage was evaluated by comparing the decrease in the removal of the target compounds in the control reactor (E) and the dark control reactor (F). Throughout the investigation, the photolysis of both AMX and CIP was negligible not exceed 4%. Our findings regarding AMX are generally in agreement with those of (Fallahizadeh et al., 2023) who confirmed that the low decomposition efficacy of AMX under LED visible light without the addition of a catalyst (photolysis process) could be attributed to the absence of oxidising species. Consequently, the degradation of amoxicillin is not significantly affected by photolysis. In addition, El-kemary et al. (2010) observed that the concentration of CIP did not alter substantially due to direct sunlight-assisted photolysis, which was determined to be only 14%.

The differences in removal efficiency between reactor (E) and (F) represented the photolysis process and reactor (F) was under dark conditions used to determine the removal of the antibiotics by the hydrolysis process. This was consistent with what was mentioned in (Habaki et al., 2023). In reactor (F) hydrolysis is the only dominant process. This is because the effect of all removal mechanisms (plant uptake, adsorption, biodegradation and photolysis) has been excluded, except for hydrolysis. Therefore, the rate of hydrolysis for AMX and CIP was roughly estimated separately based on the decrease in pollutants concentrations in the dark control reactors (F). Hydrolysis accounted for a low percentage of total AMX removal ranging between 6.2–17.8% throughout the experiment. As for the CIP, it showed results close for AMX about (9.56–17.52)% towards hydrolysis. The results are consistent with the results reported by (Abbassi et al., 2016), which were determined by the hydrolysis effect of the antibiotics in purified water. The degradation disintegration rate of ciprofloxacin was the lowest. Moreover, the impact of biological degradation and hydrolysis on amoxicillin for varying hydraulic detention times demonstrated that hydrolysis has a negligible impact on the short-term decay of amoxicillin (hydrolysis decay rate of 0.0147 d-1).

CONCLUSIONS

A lot of researchers are interested in how to get rid of antibiotics using green technologies. While phytoremediation seems like a good way to get rid of or lessen most of the Phs in wastewater, a lot of work needs to be done to find and improve the removal processes in CWs. The most important thing that this study showed was that *Ruellia simplex* can tolerate water stress without plants turning yellow or dying during the experiment. So, this plant is good enough to be used in CWs when they are grown in water. Plants that were grown in hydroponics showed that AMX and CIP did not harm them because they grew quickly. The development of a highly effective substrate with minimal secondary pollution and non-release of by-products, particularly those that are benign to the environment, to remove pollutants is a challenging task due to the absence of theoretical and experimental research on the use of CM as a low-cost substrate for Phs treatment. This study proved that a mixture of CM-WS is a promising material for removal, in addition to its benefits as a fertilizer that enhances plant growth and falls within the use of waste in treating polluted water, which seems to improve the goals of environmental sustainability. In addition, its adsorption rate is higher than traditional layers such as sand. The processes of phytoremediation, adsorption and biodegradation are the processes that control the removal for the most part, while the role of photodegradation and hydrolysis is negligible.

This study recommended using of CM-WS mixture to help plants grow by giving them nutrients, but too much of it can cause too much salt, nitrogen, or ammonia, all of which are bad for the plant. Too much of these nutrients can hurt the plant's capacity to photosynthesise and cellular respiration, which can look like burns. This is considered a limitation to the use of animal waste. For future studies, we suggest mixing CM-WS with sand so that the large amount of fertilizer does not affect plant growth, and also adding animal manure to CWs layers increases hydraulic conductivity and prevents clogging within the layers. This study suggests employing waste materials as alternative substrates in the context of waste disposal and reuse and testing the efficiency of CWs in removing pollutants by using *Ruellia simplex* and chicken manure - wood shavings (CM-WS) mixture from wastewater.

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