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COMPARISON OF CONTAMINANTS REMOVAL EFFICIENCIES IN WASTEWATER USING CONSTRUCTED WETLANDS OF SINGLE AND TWO STAGES

* Jorge I. Cifuentes [1,2]
Paris Rivera [1]
Jennyfer Paiz [1,2]
José Cortéz [1,2]
Andrea Barrera [1,2]

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Abstract

Constructed wetlands are green technologies, nature-based solutions, that use phytoremediation for the treatment of wastewater studied in domestic, industrial, and agricultural uses and products such as hydrocarbons and some emerging pollutants. The objective is to analyze the efficiency of the constructed wetlands for the removal of heavy metals, nutrients such as phosphorus, and nitrogen in wastewater treatment. This study presents the results of contaminant removal from two types of constructed wetlands; single-stage and two-stage, which determine their efficiency. The removal of COD, BOD, phosphorus, fats, nitrogen, solids and others, showed similar efficiencies for each plant, with removal ranges between 16% and 95% relative to each pollutant analyzed. As a complement, the concentration of heavy chromium VI, cadmium, and total iron were reduced and analized thlrough the UV-VIS spectrophotometric method, which from three absorbance measurements for each metal, with wavelengths of 540 nm, 228 nm, 8 nm, and 510 nm, respectively, efficiencies of 62% and 85% were found for chromium VI removal; 43% and 53% removal for cadmium; 37% and 53% removal for total iron, results according to single-stage and two-stage plants.

Keywords: heavy metals, phytoremediation, spectrophotometry, water contamination.

[1] University of San Carlos of Guatemala, Guatemala.
[2] Research Laboratory of the School of Mechanical Engineering, University of San Carlos of Guatemala, Guatemala.
* *Corresponding author:* Research Laboratory of the School of Mechanical Engineering, University of San Carlos of Guatemala, 01012, zip code: +502 4422 0831, Guatemala city. Email: 2402770580101@ingenieria.usac.edu.gt / researchnano20@gmail.com

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Introduction

A polluting element (organic or inorganic) is one whose levels are above any natural environmental parameter. High concentrations of heavy metals in untreated wastewater, such as iron, chrome, nickel, mercury, zinc, vanadium, and cadmium, give rise to reactive radicals, especially superoxides, lipid peroxides, and hydrogen peroxides that damage DNA by forming toxic compounds with proteins and amino acids (Briffa, Sinagra *et al*, 2020). Polluting elements accumulate in biological beings through absorption by dermal contact and ingestion and inhalation by water or food. If the sewage is not properly relined, it is a potential risk to aquifers and vegetables near the area; uncontrolled leachate runoff creates a potential risk to the environment (Ahmad *et al*, 2021). So any organism made up of cells can be affected at the DNA level with direct consequences on the mitochondria, lysosomes, membranes and enzymes that are inhibitory mechanisms for cell repair. The most common contamination by metallic elements, specifically those with a density greater than 5-6 g/cm^3, are elements with high density and atomic mass; the real danger is that even at low concentrations these are not biodegradable (Zaynab *et al*, 2022; Pabón et al., 2020). *Given the toxic and carcinogenic effects, chromium and cadmium are of most concern.*

Some of the existing bacterial removal mechanisms, such as physical retention, predation, oxygen release into the rhizosphere, the activity of attached bacteria in the macrophyte roots, or the ability of some helophytes to crea root exudates that inhibit bacterial growth, have been studied before (Ravindra *et al*., 2021). In free-water systems with surface flow, the hydraulic conductivity, of both the gravel bed and the rhizosphere zone, is negligible (Shingare *et al.,* 2019), and the main role of macrophytes is to provide an extra surface for the development of the biofilm on the plant submerged parts (Zhang *et al.,* 2022). In those systems with sub-surface water flow, the hydraulic conductivity of the substrate is an important design parameter to consider.

Compared with constructed wetlands, algae-based systems have been widely studied with regard to bacteria removal (Donde *at al.,* 2020). The processes involved in bacterial decay for this type of systems are completely different of those that show up in wetlands.

The polluting elements that come from wastewater, such as fats and sludge, can produce negative effects on the flora and fauna; decreased germination and enzyme activity, inhibition of photosynthesis, reduction of chlorophyll production and death by lead. In aquatic species, they reduce the number of living organisms, causing biological alterations due to the non-metabolization of metals. Wastewater contamination in aquifers produces a high concentration of biochemical oxygen demand (BOD), ammonia and hydrogen sulfide (Suchowska-Kisielewicz & Nowogoński, 2021). Most countries in the world have contamination of wastewater by fecal viruses, added to that; it is accompanied by endocrine descriptors, pharmaceutical products and pathogens. When nature and wastewater meet, biological beings are severely affected

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(Wear *et al*, 2020). Emerging contaminants such as synthetic detergents high in sodium dedecyl sulfate, widely used in cleaning and disinfection, which are highly toxic elements and can be treated by aquatic plants and microbial elements (Yasin, M. *et al,* 2021); Similarly, medications such as acetaminophen can receive similar treatment (Yang, C.W. *et al*, 2020). Factors affecting bacterial elimination in constructed wetlands include filtration, adsorption, aggregation, and metabolic activity of biofilm microorganisms and macrophytes (Kataki *at al.,* 2021). However, the main mechanisms involved in bacterial elimination in algae-based systems comprise sunlight exposure (Chambonniere et al., 2021), dissolved oxygen concentration, pH and light absorbing substances (Bea *et al.,* 2022). The objective of this study is to evaluate through a comparison of removal efficiencies which type of wetlands structure provides the greatest removal of residual water.

Phytoremediation

Constructed wetlands are processing plants based on the purification processes of natural wetlands, they have the ability to remove contaminants and nutrients present in the water. These plants are low in construction and maintenance costs, require less energy and can be implemented both on a small and large scale (Riva *et al*, 2020). Constructed wetlands carry out physicochemical and biological processes through the absorption and sedimentation of pollutants. The plants in their rhizospheric system create microbial communities used for the elimination of pollutants and the development of plants. Organisms and plants associate and even create endophytic systems (Vassallo *et al*, 2020).

In phytoremediation, plants break down contaminants through the roots, leaves, stomata, and cell walls. The most common phytoremediation is: rhizofiltration, phytostabilization, phytoextraction, phytovolitization, phytodepuration, phytodegradation and rhizodegradation; are the processes for the removal of contaminants in wastewater (Nugroho *et al*, 2021). The plants applied in constructed wetlands have the capacity to absorb contaminants in the roots, mobilize through xylem and sequester them in the rhizospheric part to be eliminated through plant residue (biomass); the main pathways are apoplastic and symplastic pathway; the filtration of contaminants occurs by adsorption and/or transport to intracellular spaces of plants. The plant must be of exponential growth, high biomass yield and ability to withstand toxicity; removals of up to 99% and a novel application of transgenic plants have been reported, more than 18 types of plants have been studied to remove heavy metals (Ali, S. *et al*, 2020). For the removal of heavy metals, there are specialized transporters or proteins with H+ between cell membranes that interact with metal ions present in the wastewater, the identified metal transporters, so far, have been classified into several families, such as ZIP, HMA, MTP and NRAMP (Yan *et al*, 2020). The role of microorganisms, of which more than 17 common types of bacteria growing in constructed wetlands for textile wastewater have been studied (Wei, F. *et al,* 2020). These favor the growth of the plant for which a granular soil is

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important, endophytes, protobacteria, bacillus, pseudomonas, cyanobacteria, archaea and others have been found, in addition to the degradation of organic matter, specific bacteria favor the translocation of toxic metals (Shahid *et al*, 2020). The combination of bacteria and floating plants of phytoremediation has proven to be effective even in the elimination of oil-diesel and hydrocarbons from 50% to 70% in a period of 90 days (Fahid, M. *et al,* 2020).

In general terms, the contaminant removal rate of wastewater treatment plants can be around 200 to 2,000 million cubic meters per day. Given human development, there are various processes for this, among which can be mentioned: membrane filtration, electrodialysis, nanofiltration, ultrafiltration, reverse osmosis, ion exchange, activated carbon, chemical precipitation, electrofluctuation, precipitation by sulfides and hydroxides, photocatalysis (Ravindra *et al*, 2014). Wetlands, under natural conditions, provide significant removal of pollutants from stormwater, wastewater, and industrial effluents. The basic process is the oxidation of organic matter, sequestration of pollutants and transport from plants that grow and thrive in wetlands hydroponically. The removal efficiency of heavy metals by constructed wetlands has been reported as high as 100% and as high as 90% for organic pathogens (Salimi & Scholz., 2021). A critical drawback of these green technologies, and perhaps their greatest challenge, is their relatively large area required for wastewater flow control. Requires an impermeable floor basin with a slight slope for continuous flow control. Constructed wetlands and their designs have been used for the removal of both emerging contaminants in agriculture (pesticides, herbicides, pharmaceutical products) with reported efficiencies of up to 100% (Tang *et al*, 2021).

The study of constructed wetlands began in the 1950s with the initial work of Käthe Seidel. The most widely used plants are floating, submerged and emergent macrophytes. Commonly applied plants in constructed wetlands are known as saprophytes, given the conditions of their environment, this plant, like Phragmites australis, have the ability to thrive in saturated soils. Most of the wetlands in Europe use plants such as Phragmites australis and Phalaris arundinacea, pseudacorus, Thypa and Cyperus spp (Stefanakis, 2020).

Wetland systems respond to other divisions according to the type of construction: Surface flow (HFS), horizontal flow (HFSS), vertical flow (HFV) and hybrid systems (SH) (Rambabu et al., 2020). The metal removal efficiency is ideal, data reported with removal rates for Zn, Cr, Ni and Pb are up to 97% (Saeed et al., 2021), while COD, BOD and suspended solids were removed at a higher rate; rate between 70 and 80%. Within the wastewater of the textile industry, physicochemical aspects such as metals Cu, Ni, Zn, Fe, Mn, and Pb were considerably reduced within the range of 70% to 90% using Phragmites australis, which maintained stability during 20 days of study (Nawaz, N. *et al*, 2020). Similarly, the removal of organic pathogens is extremely successful, with reports of up to a 57% reduction in total coliforms, 62% in fecal

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coliforms (Shukla *et al*, 2021; Torrens *et al*, 2021). The objective is to analyze the efficiency of the constructed wetlands for the removal of heavy metals, nutrients such as phosphorus, and nitrogen in the treatment of wastewater.

Materials and methods

The heavy metals analysis was carried out in the laboratory of the Research Unit of the School of Mechanical Engineering of the University Of San Carlos Of Guatemala. The equipment used consists of a VW-R UV-Vis 1600PC spectrophotometer, 10 mm glass-quartz cuvettes, pipettes, test tubes, distilled water, reagents, and equipment in general. UV-absorbance was measured in triplicate samples on a UV-1600PC spectrophotometer (VW-R). The metals under study were hexavalent chromium, cadmium, and total iron. The analysis of physicochemical parameters analyzed elements was carried out by an external accredited laboratory, the parameters analyzed were apparence, color, turbidity, pH, temperature, electric conductivity, N-NH3, N-NO2, N-NO3, total nitrogen, sedimentary solids, suspendeds solids, dissolved solids, hydroxides, carbonates, bicarbonates, total alkalinity.

For the quantification of heavy metals, reagents were used applying the specific methods of diphenyl carbohydrazide, cadion method reagent, and 1,10 phenanthroline method reagent; for chromium VI, cadmium, and total iron respectively. The standard for sample treatment and measurement belongs to the American Public Health Association (Baird *et al*., 2017). For the determination of metal concentration in the sample, the techniques of the calibration curves were consulted according to previous tests strongly adapted to this study (Sanchez-Hachair & Hofmann, 2018), with the internal standard method at known concentrations. The selected wavelengths were 540 nm, 228.8 nm, and 510 nm for chromium VI, cadmium, and total iron, respectively. The samples were collected in polyethylene bottles for pH and conductivity analysis; glass and amber for the analysis of heavy metals, physicochemical (fats, oils, and others); plastic bottles for color analysis, BOD, COD, and solids.

Design and operating conditions

The study area is under the control of the Regional School of Sanitary Engineering and Hydraulic Resources (ERIS) of the University of San Carlos of Guatemala (USAC), in this area is located a pilot plant for the study and research of wastewater treatment systems, which stil operating to date, shown in figure 1. It is made up of independent systems such as trickling filters, up-flow anaerobic reactors, biological filters, settler, two facultative lagoons, clarifying biodigester, and two constructed wetlands of single and two stages. The ERIS pilot plant is located at 14° 34' 41.6" north latitude and 90° 32' 11.8" west longitude, between 1,520 and 1,550 meters above sea level. The relative humidity of the area varies from 85% to 64% during 24 hours.

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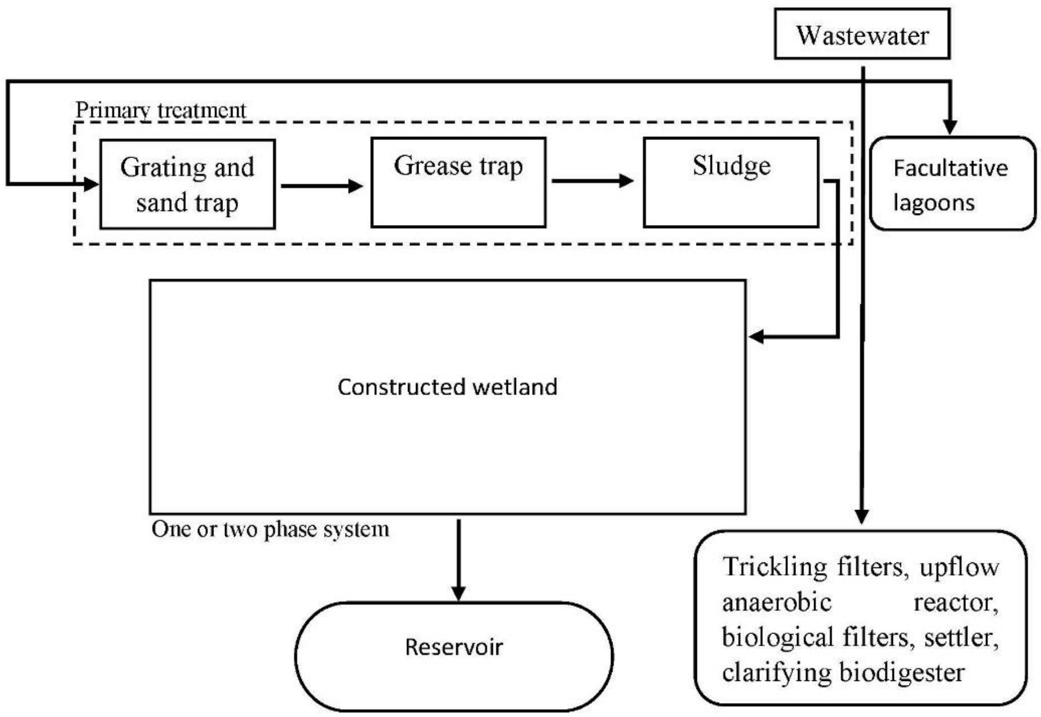

Figure 1. ERIS-USAC pilot plant flowchart

Both designs respond to an impermeable bottom, inlet channel, screens, sand trap, grease trap, and subsurface flow wetland units, with their filter media; they work under a surface and sub-surface flow process, with a hydraulic retention period of 4 days. For phytoremediation, in the single-stage plant, as shown in figure 2, *Thypa* is used, which has been used in other studies under similar conditions of altitude and precipitation; In this study, the wetland system consisted of vertical subterranean flow lagoons with a size of 2mx1mx0.8m, used for the treatment of a pig farm with removals of up to 99% of solids, organic matter and nitrogen with a pretreatment of lagoon (Denisi, 2021). *Thypa* which rests on a stone bed with a diameter between 0.07 m and 0.1 m, the separation between the plants is 0.5 m. The two-stage plant has shown in figure 3 uses a combination of *Thypa* and *Eichhornia crass*ipes that has been studied in domestic wastewater and textiles (Wei *et al*., 2020). The design flow for both cases is 0.014 L/s, and the hydraulic gradient ranges from 0.5% to 1%, with a provision of 0.2 m^3/inhabitant/day. Both plants, Figures 2 and 3 share in their entrances wastewater for residential use made up of around 200 houses.

Among the main phases is a pretreatment for the removal of coarse solids using a grid with an angle of inclination connected to a collection basket, the flow passes to a grit trap and later to the

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grease trap. The primary treatment consists of an upward concave structure to improve the efficiency in the sedimentation of solids, with a height to settle sludge. The main treatment is the two subsurface flow and surface flow units of each plant.

Figure 2. Single-stage wetland plant.

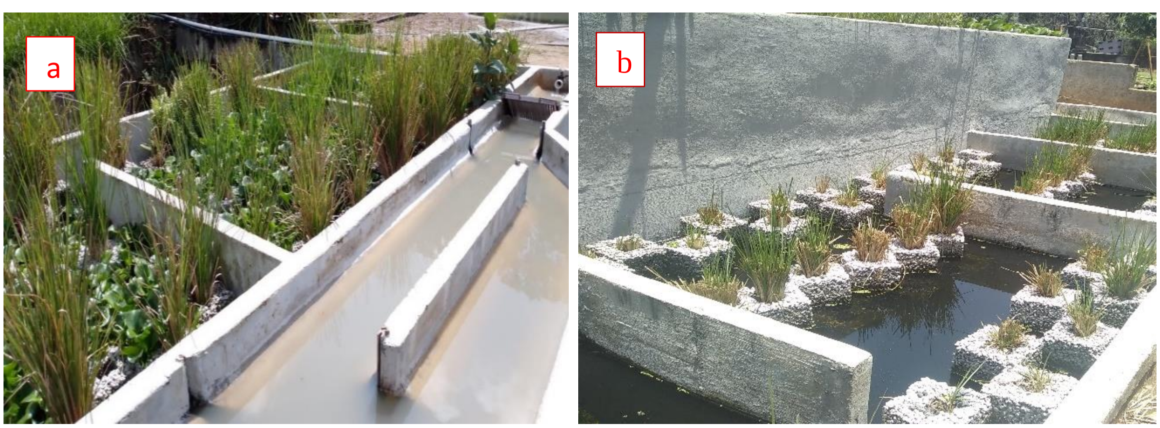

Figure 3. Wetland plant; right entrance and a) stage 1, b) left stage 2.

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Internal control analyzes of raw wastewater are established as shown in Table 1. Given the eating habit of the resident population, in accordance with the majority of the region, which is mainly based on foods high in iron, carbohydrates and others; the control of the input residual water according to physical and chemical parameters determines according to the data presented in Table 1. It should be noted that the analysis of heavy metals of the plant represents the first for treated and post-treatment residual water.

Results

The geographical position allows stable conditions in the conctructed wetland. In situ pH measurements determined an increase from 7.14 to 7.36 for the single stage contructed wetland and from 7.19 to 7.58 for the double stage pilot plant, which represents increases in the acidity of the soil. Residual water in amounts of 3% and 5% respectively. In situ conductivity measurements showed decreases of up to 57%; from 18.7 kΩ to 10.2 kΩ for one stage contructed wetland and from 18.1 kΩ to 7.7 kΩ for two stages constructed wetland.

Table 1. Typical feed wastewater conditions for both constructed wetland.

Parameter	Measure	Value
BOD	mg/l	264
COD	mg/l	495
Total coliforms	NMP/100 ml	9.80E+14
Fecal coliforms	NMP/100 ml	1.00E+14
Appearance	Aspect	Muddy
Color	Pt-Co Units	104
Turbidity	UNT	107
pH	--	6.80
Temperature	°C	22.60
Electric conductivity	S/m	660
N-NH3	mg/l	43
N-NO2	mg/l	0.05
N-NO3	mg/l	10.80
Total nitrogen	mg/l	53.85
Sedimentary solids	cm^3/l	5.00
Suspended solids	mg/l	30
Dissolved solids	mg/l	330
Hydroxides	mg/l	0
Carbonates	mg/l	0
Bicarbonates	mg/l	342
Total alkalinity	mg/l	342

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Tables 2 and 3 show the physicochemical analysis results of the accredited laboratory carried out during the semester of the current year; for the single and the doblue stage constructed wetland respectively.

Table 2. Conditions at the inlet and outlet of wastewater single-stage constructed wetland

Analysis	Input	Output	Efficiency
Color (Platinum-Cobalt Units)	137	62	55%
Biochemical oxygen demand (mg/l)	140	14	90%
Chemical oxygen demand (mg/l)	339	36	89%
COD / BOD ratio	2.4	2.6	--
Total phosphorus (mg/l)	5.3	4.45	16%
Fats and oils (mg/l)	36	<6.94	81%
Floating matter	absent	absent	--
Total nitrogen (mg/l)	27.8	10.80	61%
Sedimentary solids (mg/l)	0.5	1.0	--
Suspended solids (mg/l)	55.00	7.33	87%

Table 3. Conditions at the inlet and outlet of wasterwater, two-stage constructed wetland.

Analysis	Input	Output	Efficiency
Color (Platinum-Cobalt Units)	171	79	54%
Biochemical oxygen demand (mg/l)	139	18	87%
Chemical oxygen demand (mg/l)	367	48	87%
COD / BOD ratio	2.6	2.7	--
Total phosphorus (mg/l)	5.10	3.3	35%
Fats and oils (mg/l)	33	<6.94	79%
Floating matter	absent	absent	--
Total nitrogen (mg/l)	31.80	14.4	55%
Sedimentary solids (mg/l)	2.0	<0.1	95%
Suspended solids (mg/l)	61.25	<2.97	95%

The analysis of chromium VI, cadmium and total iron, the absorbance measurements were used in complement of calibration curves. The samples obtained were at the entrance and exit of each constructed wetland.

Figure 4 present the concentrations of each metal in inlet and outlet; the metals studied correspond to the domestic wastewater described above. The removal efficiency of each constructed wetland is also shown.

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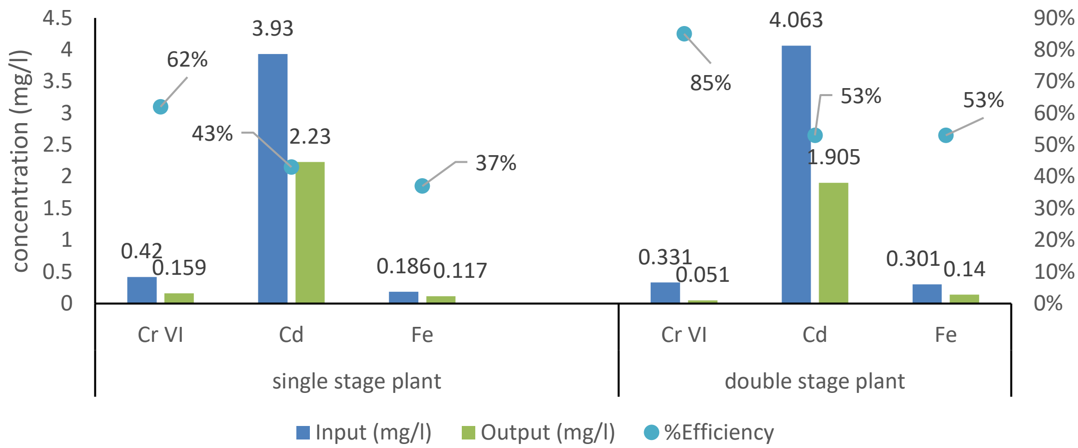

Figure 4. Comparison of the removal efficiencies of both constructed wetland.

Discussion

Even though the region has a large natural hydrological resource that makes it possible to supply the population in general, the lack of compliance with existing regulations for the protection of water has caused 90% of surface water to be contaminated with feces and other waste harmful to health (Chan and Peña, 2014). In recent years, monitoring and private studies have found that heavy metals are common in wastewater, recently detecting the presence of arsenic in the city's tap water (Chan and Peña, 2014). Drinking contaminated water increases cases of diarrheal diseases, and also has a great influence on the appearance of nutritional problems.

The fundamental role of contaminant removal efficiency, for both constructed wetland, is played by the plant species *Thypa* and *Eichhornia*, through the uptake and transport of contaminating elements in their rhizospheric system and cellular space; these elements are not consumed by the plant, but stored in its bio-residual system, also treated in the form of absorption, volatilization, filtration, and sedimentation, in addition, microorganisms also have an important influence on the efficiency of each wetland, although their analysis and characterization are outside the scope of this work. Plants and microorganisms have a symbiotic role.

The efficiency of constructed wetlands is satisfactory; studies before this one show that removal efficiencies exist in verifiable ranges; Denisi *et al*. (2021) reported removals of suspended solids, organic matter, and nitrogen in the range of 99%, 80% and 95% respectively, using typha. For the same three parameters, table 2 shows removals of between 61% and 87% for the single-stage constructed wetland and 55% to 95% for the two-stage contructed wetland. The results of this study describe that the combinations of Thypa and Eichhornia in a double stage contructed

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wetland increase the efficiency of physicochemical removals by a maximum of 20% over the combination of a single stage constructed wetland. In the sigle-stage constructed wetland, an increase in sedimentary solids was detected, probably due to operational problems in the primary treatment.

The main objective of this work was to evaluate a second stage of the constructed wetland and since the conditions are relatively stable and the same for each them, the efficiencies of the single-stage constructed wetland seem to be barely surpassed by the two-stage plant, in physicochemical removals shows that the efficiency of the two-stage plant is within the range of 1% to 20% higher than the single-stage plant; while for the removal of heavy metals it is a maximum of 20% more for a two-stage constructed wetland about hexavalent chromium. Considering previous work, such as that of Zepei Tang *et al*. (2021) on the removal of up to 99% of emerging pollutants from agricultural wastewater, suggests that the efficiency of a constructed wetland does not depend so much on the inlet channel, instead it influences more factors such as primary treatment (use of grease trap, sludge removal, ponds, etc.), and the level that can affect weather conditions and the maintenance given. On the other hand, the conditions of the feed wastewater and the number of stages seem to have little influence on the efficiency of the removal of metallic and physicochemical elements. The plants and microorganisms are also an impact factor in the removal, the *Eichhornia* used in the two-stage plant for Cd and Cr; Ali *et al.* (2020) reported removal efficiencies of 82% and 80% respectively, which is an indicator that the best efficiency of the two-stage plant comes from the combination of *Thypa* and *Eichhornia* and does not depend as much on a second stage, although a deeper analysis of the microbial elements is required to confirm it.

In the removal of heavy metals, Cd, Cr VI, and Fe, the removal percentage is identical to the one previously described, with an efficiency improvement of between 10% and 23% for the two-stage constructed wetland. Both share the same operating conditions.

Given that the contructed wetlands share the input of residual water, the conditions at the beginning of each one vary considerably, in this sense it is to be assumed that the treatment conditions before phytoremediation, referring to the grease trap and mud trap, are important. For the proper functioning of the constructed wetlands, even though they share similar designs, the physical conditions of the two-stage constructed wetland are in better condition. It can be summarized that the detection of contaminants is relatively consistent in both, although during the measurement it was not considered where to find the maximum possible concentrations, as occurs in the accumulation of sludge. The two-stage constructed wetland has the best efficiencies for contaminant removal, with up to 85% removal of hexavalent chromium compared to 62% for the single-stage constructed wetland.

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The application of green technologies such as constructed wetlands in small communities can represent a significant improvement in preservation, especially of aquatic resources; given that the contaminant removals, of an organic or inorganic nature, are presented in percentages of up to 99%; Although it is a technology that requires a large area for its operation, its automated operation, maintenance costs, and construction costs make it attractive for its application. Long-term performance and operational sustainability are the great challenges of constructed wetlands, where operational times, hydraulic retention, flow mode, and pretreatment design all affect plant stability.

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