

# Enhanced heavy metal removal by wetland vegetations and its significance for vegetation-activated sludge process configuration

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#### ABSTRACT

Under the present investigation, removal capacities and influential factors of four heavy metals (HMs) (Cu, Pb, Cr, and Ni) by three wetland vegetations, i.e. *Canna indica, Dracaena sanderiana, Cyperus alternifolius*, were conducted to assess their feasibility and reliability to collocate into an ecological vegetation-activated sludge process (V-ASP) for HMs contained wastewater treatment. The maximum specific Cu, Pb, Cr, and Ni removal rate of *D. sanderiana*, *C. indica*, and *C. alternifolius* was 17.6, 6.39, 19.09, and 7.05 mg/kg WW-d, respectively. Co-existence of organic and nitrogenous pollutants and their content fluctuation affected significantly on Cu, Pb, and Ni removal efficiency, and moderately on Cr removal. A weak alkaline (7.5–8.0) in bulk favored Cu and Pb removal, while a weak acidic (~6.5) was beneficial to Ni elimination. Vegetation roots had a much higher HMs concentration than their stems and leaves, and continuous HMs accumulation within vegetation decreased its photosynthesis rates, and made various morphological changes, although which would be recovered under proper conditions. Evidences of stable HMs removal by proper vegetation collocation give direct guideline for V-ASP configuration, which have profound significance for its practical application.

*Keywords:* Heavy metals removal; Wastewater treatment; Wetland vegetations; Vegetation-activated sludge process

#### 1. Introduction

Heavy metals (HMs) contamination in aquatic ecosystem causes serious threaten to aquatic biodiversity, and results in severe health risk to human beings, mainly due to their non-degradable and persistence toxic nature [1,2]. Several physicochemical methods have been used to treat HMs contaminated wastewater, including adsorption, chemical precipitation, ionexchange, and membrane separation, most of which, however, are costly, energy intensive, and metal specific [3]. Constructed wetland (CW) is an ecological wastewater treatment process that can effectively remove suspended solids, organic substances, nitrogen, and phosphorus compounds and bacteria [4]. Recently, more and more concerns on CW capability and reliability in removing micro-pollutants, particularly HMs, were raised and many researchers found that CW could remove a certain amount of HMs from wastewater, contaminated soil, and sediments [5,6], as that plants have great potential to accumulate HMs

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inside their issues. Nevertheless, CW has its own shortcomings during practical application, including a rather low surface loading rate and large footprint requirement, a long hydraulic retention time (HRT), and a strenuous plant management, making it low suitability for decentralized wastewater treatment with small and fluctuated flow-rate.

The requirement of decentralization wastewater treatment brings about an increasing development of ecological wastewater treatment processes, such as living machine system and vegetation-activated sludge process (V-ASP), both of which possess numerous merits, including high micro-pollutants removal efficiencies, small footprint, and short HRT. As one major constitution, vegetation offers potential capabilities in HMs removal via filtration adsorption, cation exchange, and through plant induced chemical changes in its rhizosphere. Notwithstanding, the feasibility and reliability of V-ASP process for HMs contained wastewater has to depend on the vegetation species, co-existed biomass characteristic, and wastewater quality. However, there are a few studies focused on the V-ASP configuration and application for HMs contained water treatment, up to now.

Therefore, in the present work, three wetland vegetations, Canna indica, Dracaena sanderiana, and Cyperus alternifolius, were employed to remove various HMs, i.e. Cu, Pb, Cr, and Ni, from synthetic wastewater, accordingly to assess their suitability and reliability for V-ASP configuration. The experimental study was carried out: (i) to evaluate the HMs removal capability of wetland vegetations under different impact factors that have to be encountered during V-ASP configuration, (ii) to examine the influence of HMs accumulation in wetland vegetations, which affected V-ASP performance, and (iii) to investigate related significance for V-ASP allocation mode with wetland vegetations. The experimental results will give direct evidences on HMs removal by wetland vegetations, and hence further broaden V-ASP application.

### 2. Materials and methods

#### 2.1. Experimental design and operation

Three wetland vegetations, *C. indica*, *D. sanderiana*, and *C. alternifolius*, were selected to assess their heavy metal removal capacities for four HMs ( $Cu^{2+}$ ,  $Pb^{2+}$ ,  $Cr^{3+}$ , and  $Zn^{2+}$ ) from synthetic water. Sixteen identical plastic buckets (working volume of 1 L, Polyethylene) were operated side by side in the batch–mode to evaluate HMs removal efficiency and kinetics. The solution in the reactors was well suspended by the paddles mixing at 30 rpm. The reactors were fed with

synthetic HMs solution that contained 1 mg/L single HM (Cu<sup>2+</sup>, Pb<sup>2+</sup>, Cr<sup>3+</sup>, and Zn<sup>2+</sup>), co-existed carbon source, and necessary nutrients for vegetation growth. One vegetation sapling was immersed into reactor liquid. More than 20 mL liquid samples were taken in 2, 4, 8, 18, and 24 h to measure its HMs content and to evaluate HMs removal rate. All of vegetation saplings were gently weighed to obtain its wet weight.

The influence of nutrients loadings and pH was carried out in series of semi-continuous hydroponics experiments by using 48 identical plastic buckets (working volume = 1 L). An individual vegetation sapling was installed into bucket with its root completely immersed into the solution. The selected vegetation had rather similar shape, size, and weight to make the results comparable. Around 1 mg/L individual heavy metal was dosed into wastewater to simulate hydroponic solution. Every experimental trial was carried out for about one month, which was more than 70 HRT of the system to examine system stability. Three different experimental conditions were designed according to various nutrients loading rates (Table 1), and Run I was operated as a blank. The bucket bulk solution was discharged completely and re-filled with fresh feeding water in every 3 d. Bulk solution components were regularly monitored to evaluate HMs removal performance. As for the experimental trials on the effect of pH, the composition of feeding wastewater was kept as 8.0 mg  $NH_4^+$ -N/L, 2.0 mg TP/ L, and 17.5 mg COD/L, and the pH was maintained at 5.5, 6.5, 7.5, and 8.0, respectively.

A long-term HMs removal experiment was conducted in a continuous stirred tank reactor (CSTR) with a working volume of 170 L (L × W × H = 120 cm × 58.5 cm × 25 cm) for one month. A constant inlet flow-rate of 420 L/d was kept to maintain a HRT of 10 h, and the feeding water comprised of mixed HMs of around 0.5 mg/L Cu, Pb, Cr, and Ni.

#### 2.2. Wastewater preparation and analysis

The feeding wastewater for experimental tests was collected from a discharge point of an industrial zone located in Pinshan district, Shenzhen, China. The major compositions, including pH, COD,  $NH_4^+$ -N, and TP, were measured regularly, whose concentration was ranged at 7.41 ± 1.37, 32.09 ± 13.51, 16.59 ± 8.34, and 4.22 ± 1.38 mg/L, respectively. HMs i.e. Cu, Pb, Cr, and Ni, with pre-determined content were dosed in term of cupric chloride dihydrate (CuCl<sub>2</sub>·2H<sub>2</sub>O), lead nitrate (Pb(NO<sub>3</sub>)<sub>2</sub>), chromium chloride hexahydrate (CrCl<sub>3</sub>·6H<sub>2</sub>O), and nickel chloride (NiCl<sub>2</sub>) (reagent grade chemicals, Aladdin, Shanghai, China), respectively, into wastewater to prepare synthetic HM

|     | Operational condition            | pН              | NH <sub>4</sub> <sup>+</sup> -N (mg/L) | TP (mg/L)       | COD (mg/L)        |
|-----|----------------------------------|-----------------|--|-----------------|-------------------|
| I   | With HMs while without nutrients | $7.25 \pm 1.05$ | 0                                      | 0               | 0                 |
| II  | With HMs and nutrients           | $7.38 \pm 0.65$ | $7.98 \pm 5.55$                        | $2.08 \pm 0.4$  | $17.49 \pm 8.51$  |
| III | With HMs and nutrients           | $7.41 \pm 1.37$ | $16.59 \pm 8.34$                       | $4.22 \pm 1.38$ | $32.09 \pm 13.51$ |

Table 1Experimental design and operational conditions in the batch-mode experiments

polluted wastewater [7]. Wastewater samples were collected and then immediately analyzed its COD,  $NH_4^+$ -N, and TP according to the standard methods (Chinese Standard Methods for the Examination of Water, 2002) after gentle filtration (0.45 µm, Millipore). The pH was measured by a digital pH meter (PB-10, sartorius, Germany). Four HMs concentrations before and after experimental tests were quantified using ICP-OES (optima 8000, PE, USA).

# 2.3. Vegetation parameters estimation and their tissues analysis

The activity of vegetation was indicated in term of photosynthesis rate (Pn,  $\mu$ mol CO<sub>2</sub>/m<sup>2</sup> s) that was examined by photosynthetic and transpiration Analyzer (SY-1020, China). Upon steady-state phase reached, HMs content and distribution profile within vegetation tissues were estimated after its tissues dry-weight measurement and acid digestion [8]. Vegetation tissues were dried under 80°C for 24 h to quantify its dry-weight. A low temperature was used to avoid vegetation thermal decomposition, where 1 g homogenized dry sample was digested with 1 M nitric acid. Afterward, the digestion solution was neutralized using 1 M NaOH, and its HM concentration was determined. All of analyses were carried out at least for three times to make the results convincible.

### 3. Results and discussion

# 3.1. HMs removal rate by various wetland vegetations

The selected wetland vegetations had varied HMs removal capabilities during experiments (Fig. 1), and an obvious elevation trend of HMs removal efficiency with the experimental progression was observed for every vegetation. Analysis of metal concentrations with experimental time suggested that the HMs removal efficiency with operational time displayed two-stages pattern, including an initial short-term rapid HMs removal stage and a long-term slight HMs removal rate stage. For instance, *C. alternifolius* could remove up to 80% Pb<sup>2+</sup> in the first 4 h, after which the removal



Fig. 1. Removal efficiency and removal rate of Cu, Pb, Cr, and Ni by three vegetations.

efficiency reached 93% in next 20 h. In addition, the wetland vegetation had varied removal efficiencies for different HMs, e.g. the maximum Cu<sup>2+</sup> removal efficiency above 82.0% was obtained by C. alternifolius, followed by 79.2% of D. sanderiana and 69.8% of C. indica. Meanwhile, the maximum Pb<sup>2+</sup>, Cr<sup>3+</sup>, and Ni<sup>2+</sup> was 93.4, 86.5, and 75.1%, respectively, which was achieved by C. alternifolius. However, considering the differences in vegetation wet weight (WW), D. sanderiana displayed the highest specific Cu<sup>2+</sup> metal removal rate of around 17.6 mg  $Cu^{2+}/(kg WW-d)$ . The maximum specific Pb<sup>2+</sup>, Cr<sup>3+</sup>, and Ni<sup>2+</sup> removal rate was 6.39, 19.09, and 7.05 mg/(kg WW-d) that was obtained by C. indica, C. indica, and C. alternifolius, respectively. From the viewpoint of vegetation suitability for V-ASP configuration, it is reasonable to think that the projective area vegetation occupied seems to be an essential parameter, as that the surface area of ASP tank is limited. Thus, the maximum specific  $Cu^{2+}$ ,  $Pb^{2+}$ ,  $Cr^{3+}$ , and Ni<sup>2+</sup> removal rate against vegetation projective area was 17,590, 8,390, 19,090, and 15,600 mg/(m<sup>2</sup>-d) for D. sanderiana, C. indica, D. sanderiana, and C. alternifolius, respectively. Ni<sup>2+</sup> is deemed to be one of the most difficult metals to be removed from water due to the result that D. sanderiana and C. indica could only remove more than 30 and 20% Ni after 24 h. Among

these vegetation used for HMs removal experiments, *C. indica* and *D. sanderiana* were highly efficient in Cr and Cu removal, respectively, and *C. alternifolius* had the stable removal efficiencies, especially during experimental initial phase.

The wetland vegetations selected herein showed quite higher HMs removal efficiency in contrast to the aquatic plants did [9]. As there were several HMs accumulative stages in vegetation, including immobilization and uptake from the contaminations, compartmentalization and sequestration within the root, xylem loading and transport, distribution between metal sinks in the aerial parts and sequestration, and storage in leaf cells [7,10], wetland vegetations might have much higher capability in HMs immobilization and uptake, since that they subjected to contaminants for numerous of generation. Besides, they also could luxuriant growth in HMs and nutrient rich media, and therewith to enlarge biomass accumulation for HMs adsorption.

### 3.2. Effect of feeding water compositions and pH

# 3.2.1. Co-existed organic and nutrients components

Wetland vegetations displayed various removal performances in removing different HMs when solution nutrients composition changed (Fig. 2). As for *C. indica* in Run I without nutrients addition, its Cu<sup>2+</sup>

removal efficiency and rate averaged at 76% and 22.5 mg/kg WW-d, respectively. In contrast, after extra nutrients and COD dosage, Cu<sup>2+</sup> removal efficiency declined from 76 to 60% in Run II and to below 55% in Run III. Similar trend was also observed in C. alternifolius. Considering the major pathways for Cu removal or accumulation in vegetation, initial metal immobilization onto roots is a key step [11], and occurrence of organic and ammonia compounds inevitably enriched and accumulated biomass around vegetation roots, which would occupy activated immobilization effective site and then to constrain HMs removal efficiency [12]. In contrast, with an increased concentration of organic and nutrients, D. sanderiana showed an elevating trend in Cu removal efficiency and rate. One possible reason for this discrepancy may be the fact that *D*. sanderiana was capable of utilizing wastewater compositions to enhance Cu accumulation. Besides, according to the reports of Paredes et al. [13], co-existed organic and nutrients would enrich Rhizobacteria in the vicinity of D. sanderiana root, which could luxuriously immobilize Cu from water.

Occurrence and concentration increasing in organic and nutrients substances in feeding solution performed insignificant impact onto Pb removal efficiency by *C. indica* and *C. alternifolius,* as illustrated in Fig. 2. The rather stable or slight fluctuation of Pb removal may attribute to high Pb accumulative capabilities of wetland vegetation and relative low Pb concentration



Fig. 2. Influenced of feeding water organic and nutrients on HMs removal efficiency and specific HMs removal rate.

applied, since that several reports reported that wetland vegetation could accumulate up to 36 mg Pb/g WW [14]. However, wastewater organic and nutrients co-existence elevated Pb removal efficiency by *D*. *sanderiana* from 62% in Run I to around 85% in Run II and III, while its specific Pb removal rate enhanced from 3.95 to 5.77 mg Pb/kg WW-d. Similar to that in copper removal, biomass enrichment around *D*. *sanderiana* root and cultivated *Rhizobacteria* might take major responsible.

Although chromium is a non-essential element for wetland vegetations growth, it was often detected in vegetations tissues, evidencing that it was one easyuptake HM. After a long-term incubation with or without organic and nutrients, both of *C. indica* and *D.* sanderiana showed a quite stably high Cr removal efficiency, averaged at 81 and 83%, respectively. Co-existed COD, PO<sub>4</sub><sup>3-</sup>-P, and NH<sub>4</sub><sup>+</sup>-N and their concentration variation had slight influence onto Cr removal. A similar trend was also observed in C. alternifolius, whose Cr removal rate was independent of wastewater compositions. However, continuously decrease in Cr removal efficiency from 93 to 67% in day 33 was observed, which may be due to the detrimental impact of accumulated Cr within vegetation on its growth and reproduction [15].

All of three wetland vegetations displayed rather low Ni removal efficiency, ranged between 10 and 38%, or even below. Occurrence of organic and nutrients in feeding solution has negative impact onto Ni removal performance, resulting to a continuous decline of Ni removal rate that was as low as 0.8 mg/ g WW-d. Negative impact of Ni accumulation on vegetation growth or reproduction appeared in day 10, leading to vegetation yellowing and chlorosis of leaves and root shedding. In addition, biomass growth and biofilm formation around vegetation root were also very limited, as a result of accumulative toxicity from Ni.

# 3.2.2. Feeding water pH

As an important factor regulating HMs ionizing pattern and affecting vegetation immobilization capability, pH of feeding water showed eventually important role in HMs removal efficiency and rate [16]. Four initial pH, 5.5, 6.5, 7.5, and 8.0, were kept during the experiments to evaluate their effect on HMs removal. As shown in Fig. 3, wetland vegetations showed rather stable Cr removal efficiency that has a weak relation with pH changes. In comparison, a weak alkaline (7.5–8.0) solutions favored Cu and Pb removal, while a weak acidic (~6.5) circumstance was beneficial

to Ni accumulation by all three vegetations. Fluctuation of pH from neutral to acidic or alkaline changed the HMs partition profiles between solid and liquid phase, and also disturbed HMs immobilization and biomass activities, to affect HMs removal and accumulation profiles significantly, which would result to a sharp decrease in Cu removal by C. indica and C. alternifolius, and of Ni removal by C. indica, D. sanderiana, and C. alternifolius, and also lead to a gentle decrease in Cu removal by D. sanderiana and of Pb removal by C. indica and C. alternifolius. These changes recovered to some extent after more than 14 d incubation, such as Cu removal by D. sanderiana, Pb removal by C. indica and D. sanderiana, which could be changed to original level again that attribute mainly to gradual adaption of vegetation and biomass to pH changes. Notwithstanding, some vegetation reached a new stable low-removal efficiency, such as Ni removal by D. sanderiana and C. alternifolius. As that one major way for HMs removal is the metal ions adsorption, two possible reasons may be addressed from the views of vegetation immobilization and adsorption or uptake, first was that a low pH resulted to great number of  $H_3O^+$  ions that may compete with metal ions for exchange sites, which determined the HMs uptake capacity [9], while the other was that HMs with positive charge would be preferentially precipitated in a high pH solution [11]. In addition, a low pH elevated concentrations of H<sup>+</sup> ions within acidic environments that would cause direct toxic threat to plant species and interfere with nutrient uptake mechanisms [17], by constraining the efficiency of the H<sup>+</sup> efflux pump within the roots, and by inhibition of nitrogen and calcium uptake [18].

#### 3.3. HMs accumulation and its impact on vegetation

Pronounced HMs accumulation within wetland vegetation was observed after HMs translocated from roots to leaves. Fig. 4 showed copper concentration in different parts of three vegetations after 33 d incubation, and it was observed that the Cu concentrations in day 33 were more than 10 times of the initial values of C. indica and D. sanderiana. Accumulation of the Cu in the root of C. indica, D. sanderiana, and C. alternifolius was about 4,330, 1,990, and 1,485 mg Cu/g-dry weight, respectively. As an essential micronutrient for normal vegetation metabolism, excessive Cu accumulation in vegetation is rational that depended on vegetation own properties. The extent of Cu accumulation observed in the present work was relatively high compared with previous results, e.g. Zaved et al. [19] found the maximum Cu accumulation in duckweed



Fig. 3. Heavy metal removal efficiency influenced by initial pH of feeding water.



Fig. 4. Copper concentrations in vegetation roots, stems, and leaves after 33 d incubation experiment.

(*L. minor* L.) was 3,400 mg/g dry-weight that related closely with the Cu concentration in wastewater, and Mishra and Tripathi [20] observed that the highest Cu concentrations in *Pistia stratiotes L. Spirodela polyrrhiza W. Koch*, and *E. crassipes* was 875, 186, and 2,750 mg/kg-dry weight, respectively.

The vegetation root had much higher Cu concentration than those in its stems and leaves. The Cu concentration in *C. indica* root was more than 17.6 times higher than that in its stem, and above 188 times than that in its leaves, while those of *D. sanderiana* and *C. alternifolius* were 1.8 and 29.6 times, and 22.8 and 39.2 times, respectively. These phenomena were in agree-

ment with the results of Mishra and Tripathi [20], where they found the maximum quantity of element contaminant was always contained in plant roots. The major reason for this metal partition results is the fact that vegetation have self-regulation capability to allocate micronutrients (essential or non-essential) concentrations and distribution in different parts to prevent intoxication by adjusting metal influx as well as efflux transporters.

HMs accumulation inevitably led to significant physio-bio-chemical responses to its tissues and growth profiles. One key parameter to evaluate vegetation activities, photosynthesis rate (Pn), before and after HMs accumulation, was compared in Fig. 5. The results revealed that the Pn value decreased after HMs accumulation, indicating that the growth and reproductive rates of wetland vegetation declined significantly. Besides, there were some morphological symptoms occurred during the incubation time, e.g. chlorosis of *C. alternifolius* leaves, although its stem was not affected. In contrast, *C. indica* and *D. sanderiana* showed relative constant morphological symptoms during experimental time, even their Pn value declined.

# 3.4. Feasibility of HMs removals by wetland vegetations working as V-ASP process

Stable HMs removal, elimination, and accumulation by wetland vegetation were evidenced in series of batch and continuous tests. As for the long-term



Fig. 5. The photosynthesis rates before and after HMs accumulation (*CI*, *DS*, and *CA* indicate *C*. *indica*, *D*. *sanderiana*, and *C*. *alternifolius*, respectively).

examination, where a total mass up to 46,800 g vegetations exposed to target HMs, vegetations removed more than 92.01% Cu, 90.08% Pb, 92.22% Cr, and 44.93% Ni, whose removal efficiency kept relatively stable (Fig. 6). The stability of HMs removal by wetland vegetation was also confirmed by other researchers. Take *C. indica* as an exemplification, it has been largely used in CW for the treatment of different effluents [14], and it could remove HMs, including Cu, Pb, Cr, and Zn from industrial wastewater effluent [10,13]. Notwithstanding, as for V-ASP process for HMs-contained wastewater treatment, a reasonable vegetation collocation for a stably excellent decontamination effectiveness closely related with wastewater compositions and HMs species.

A V-ASP process involved C. indica, D. sanderiana, and C. alternifolius can be used for HMs elimination



Fig. 6. Removal efficiency of four HMs in CSTR.

from several kinds of wastewater. Firstly, as for the industrial wastewater, where malfunction problem may cause untreated or substandard treated wastewater discharge into water body. Secondly, continuous discharge of treated effluent contained standard HMs content may enrich HMs in sediment when an industrial factories located in a riverside, which has potential of overproof risk in downstream [1,2]. Moreover, as point source inputs continually addressed, there are a great fraction of HMs burden to local waterways attribute to non-point sources, especially including initial rainfall runoff from industrial and mine district, which carried considerable HMs to receiving water. Nevertheless, apart from these possible ways of V-ASP that may be used, it is also believed that there are other kinds of wastewater treatment should be treated by V-ASP process, especially for decentralized wastewater.

While the wetland vegetation allocated into V-ASP process, its removal capacity was estimated according to the experimental results. For an example of sequencing batch reactor (SBR) combined with C. indica to treat more than 5,000 m<sup>3</sup>/d wastewater, the reactors have about 720 m<sup>2</sup> surface area with an 8 h HRT and a 3 m water depth. It is reasonable to assume the plant density is 100 cm<sup>2</sup>/vegetation, and each C. indica has an average wet weight about 3 kg. Accordingly, more than 54,000 kg vegetation can be installed into SBR, and thus its Cu removal capability is 428 g Cu/d (7.93 mg Cu/Kg C. indica-d). Considering to satisfy the discharged effluent standard (<0.5 mg/L) in China, the maximum Cu concentration in influent is above 0.8 mg/L, which is a typical value of an initial storm-water runoff [21].

#### 4. Conclusions

Wetland vegetations have various HMs removal efficiencies and rates. The maximum specific Cu and Cr removal rate was 17.6 and 19.09 mg/(kg WW-d) that was obtained by D. sanderiana, and C. indica, respectively. Feeding water compositions and pH affected HMs removal efficiencies. The Cu removal efficiency and rate of C. indica and C. alternifolius decreased as coexisted COD, PO<sub>4</sub><sup>3-</sup>-P, and NH<sub>4</sub><sup>+</sup>-N concentrations increased. In contrast, wastewater substances performed insignificant impact onto Pb removal by C. indica and C. alternifolius, and resulted in an increasing trend of the HMs removal efficiencies by D. sanderiana, and also showed slight influence onto Cr removal. Weak alkaline (7.5-8.0) solutions favored Cu and Pb removal, while weak acidic (~6.5) was beneficial to Ni accumulation into wetland vegetation. Cr removal efficiency had slight relation with pH changes. As for a long-term examination, a stable Cu, Pb, and Cr removal, elimination, and accumulation by proper vegetation collocation were evidenced. In a view of V-ASP capacities estimation, a SBR combined with *C. indica* could completely purify more than 5,000 t/d initial stormwater runoff contained up to 0.8 mg HMs/L to satisfy discharged effluent standard in an eight HRT, which give a promising prospective to V-ASP application.

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