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Proficiency of Brahmi (Indian Pennywort) *Hydrocotyle asiatica* in One-pot Secondary and Tertiary Treatment of Sewage in SHEFROL® System

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ABSTRACT

The Indian pennywort *Hydrocotyle asiatica* (synonym *Centella asiatica)* is a commonly occurring plant which is native to Asian wetlands. It is a keystone medicine in the Ayurvedic system where it is referred as brahmi. Yet it grows much more in nature than can be put to use and is often found where it is unwelcome such as in drainage channels and paddy fields, and is considered a weed. In this paper we report studies which show that *H. asiatica* can very efficiently treat sewage of varying strength when used as the main bioagent in SHEFROL® bioreactors. It affects primary and secondary treatment in terms of suspended solids, BOD and COD removal as also significant tertiary treatment *vis*-a-*vis* nitrogen and phosphorus removal, in a single process unit. This raises the prospect that monumental volumes of sewage that are discharged untreated in most developing counties could be phytoremedied with *H. asiatica* because the SHEFROL® technology is itself very inexpensive and clean-green, yet simple and efficient.

INTRODUCTION

Challenges associated with sewage treatment: India and China are not only the two most populous nations of the world, they are also among the most insanitary ones, with ca 80% of the estimated 270 billion litres of the sewage generated by them being discharged untreated every day in water-bodies and on land (Abbasi & Abbasi 2018, Narain 2012, Tauseef et al. 2013a). This has caused very seriously pollution of most of the rivers and lakes of these countries. Also monies worth billions of dollars are spent every year on combating diseases of humans and damage to ecosystem services that this pollution causes (Narain 2012). The situation in most other developing countries is no different (Abbasi & Nipaney 1986, Narain 2012). Only a few parts of major cities and towns are serviced by centralized sewage treatment plants, while most households are either dependent on septic tanks-which generate highly putrid effluents and are unsuitable except if used in situations when the number of households per unit land area is low-or have no sewage treatment facility at all. Such situations prevail in most suburbs and rural areas, and in all slums (Narain 2012, Tauseef et al. 2013a).

Between 2009 and 2015 sewage generation in Indian cities increased by 62% while the sewage treatment capacity in the corresponding period increased by only 19% (Narain 2016). In absence of any effective measure, this trend will persist and ever greater volumes of sewage will go untreated as the population rises and rises, thereby burdening the environment more and more. As of now well over 62 billion litres of untreated sewage is released into Indian rivers daily, which amounts to over 3 trillion litres annually (Narain 2016). This input is also set to increase substantially.

The pollution of other wetlands, and stretches of land, due to release of raw sewage, is equally enormous (Abbasi et al. 2013). The situation is very similar in all other developing countries, and reflects the mind-blowing scale of the sewage treatment challenge that is faced by the majority of the world's population.

This backdrop reflects that development of cheaper and greener technologies for wastewater treatment forms one of the greatest challenges for environmental technologists. There is an even more pressing need to develop wastewater treatment systems which are suitable for small-sized neighborhoods, peri-urban areas, and rural areas which lie outside the coverage of centralized wastewater treatment facilities. In order to be viable, such systems must have the following attributes: (i) should be low-cost; (ii) should be easy to install; (iii) should be easy to operate and maintain; (iv) should generate little or no waste of their own; and (v) should be economical as well as technically viable at very low to medium scales.

We have developed a bioreactor which promises to contribute substantially in meeting this challenge. The reactor harnesses the special ability of the roots of vascular plants to enable physical, chemical, and biological treatment of biodegradable wastewater in a single step. The harnessing is achieved in specially designed narrow channels in which selected species of macrophytes are stocked to capacity. The wastewater is then made to flow though the channels as a sheet of water tall enough to just cover the plant roots. On one hand, this provides the largest number of densely packed roots possible (for that species of plant) per unit reactor space. On the other hand it enables plants to remain erect by themselves without any extra means of support. The third major advantage is that the wastewater comes in contact with only that part of the plant which is the hub of the processes leading to bioremediation (Abbasi & Abbasi 2010 a,b, 2011a). This maximizes treatment efficiency for given contact time. Fourthly the system enables good agitation and consequent aeration of wastewater without having to consume materials and energy for providing aerators. There are various other advantages linked to SHEFROL® design which have been detailed in the patent document (Abbasi et al. 2012).

Among the various specialities of SHEFROL® is that it needs no soil or any other means to anchor the plant roots, yet makes it possible to utilize not only free-floating macrophyte species like water hyacinth, *Salvinia* and *Pistia* but also those species which, in nature, grow rooted in soil both aquatic and terrestrial. This puts SHEFROL® to great advantage compared to the presently most widely used macrophyte-based wastewater treatment technology, revolving round 'constructed' or 'artificial' wetlands. In this technology hydraulic retention times (HRTs) of several days are needed in comparison to 6 hours or lesser needed in SHEFROL®. This makes SHEFROL® several times faster.

Water pennywort *Hydrocotyle asiatica*, also named *Centella asiatica,* is an aquatic, perennial, herb native to South-east Asia and the Indian sub-continent (Ghadira & Goetz 2013). It is used as food in some regions, especially Mayanmar and Sri Lanka (NurulIzzah et al. 2012, Siew et al. 2014) and is a major Ayurvedic and Homoeopathic medicine (Maulidiani et al. 2014). In the Ayurvedic system it is named 'brahmi'(along with *Bacopa monniera)* and is an apex remedy which is believed to enhance mental faculties. It is also known to relax the central nervous system and promote good sleep. But all these uses consume only a tiny fraction of *H. asiatica* that grows in nature and in many situations the herb moves in where it is not welcome, turning into a weed. Such situations principally occur in irrigation canals and drainage channels where the presence of *I. aquatica* reduces the conduit's water carrying capacity. It also slows down the flow of water, prompting spillage when inflowing water keeps coming at a higher rate than is discharged by the conduit (Abbasi 1997, Abbasi & Abbasi 2011, Ghedira & Goetz 2013).

Considering that *H. asiatica*, is not only easily available free of cost, but there is also a need to find ways of utilizing it so that it can be harvested more frequently, thereby exercising control over its unwanted growth, the present study has been undertaken. *H. asiatica* has been explored as the main bioagent in bench-scale SHEFROL® reactors for treating sewage. The performance was explored indoor as well as outdoor.

MATERIALS AND METHODS

Gathering of *H. asiatica* and its acclimatization: For stocking into SHEFROL® channels, plants of *H. asiatica* were collected from marshy areas situated near the Pondicherry University campus. Healthy adult, specimens with well-grown roots were chosen for the purpose. They were washed with tap water to free the clinging invertebrates as also to remove blobs of muck. They were stocked in SHEFROL® channels to achieve a biomass density of 3 kg/m^2. For 3 days they were continuously fed a nutrient solution (Table 1) to allow them to get acclimatized. The feeding of sewage diluted to desired level of COD was then commenced.

Setting up SHEFROL®reactors: Bench-scale SHEFROL® systems were set up as depicted in Fig. 1. After lead-up studies on channels of different dimensions, a width of 15 cm and a depth of 10 cm was found to best suit the system as it helped the plants, when densely packed, to remain erect without any support media or anchor. The dimensions also minimized short circuiting and maximized agitation as the wastewater flowed through the plant roots.

The channels which were 4 m long, were fabricated with aluminium foil of 28 gauge thickness and lined with HDPE (high density polyethylene) sheets to prevent rusting and leakage.

In the initial stages of the work the wastewater was fed at the receiving end of each channel using plastic buckets which had a tap fixed near their bottom. The buckets were so positioned that they released the wastewater at the top end of the entrance to each channel. The treated water was allowed to flow out from near the bottom at the exit at end of each channel. In this manner the flow was maintained by utilizing the liquid head and without the need of any pump. The hydraulic retention time (HRT) for any given depth of channel operation was controlled by appropriately positioning the influent release level and the rate of inflow-

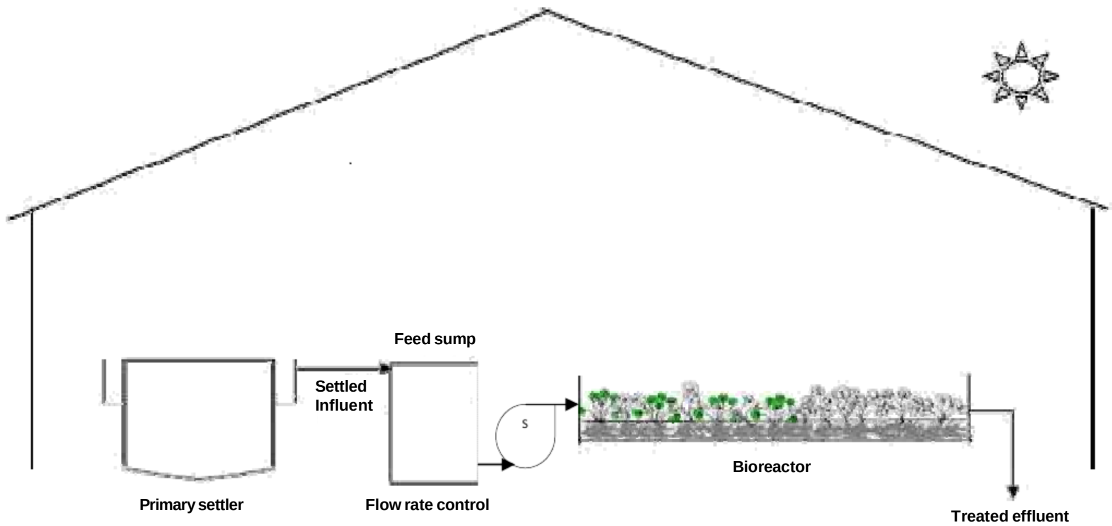

Fig. 1: Schematic of the SHEFROL® bioreactor.

Fig. 2: Indoor SHEFROL®channels stocked with *H. asiatica.*

outflow. After the initial trials, and for the sake of generating utilizable basic data, the bucket-based feed arrangement was substituted with peristaltic pumps. The latter delivered precisely controlled flows and enabled accurate maintenance of the HRTs.

Arrangement of artificial lights for indoor studies: As the diffused sunlight that lights up indoors during day-time is not adequate to support growth of most plants, we had to augment it by simulating those portions of sunlight that are needed by plants (Fig. 2). These portions of wavelengths required in photosynthesis, form about 37% of the sunlight energy (NASA 2009, Ponni 2012). We used a combination of twelve 40W fluorescent lamps and three 100W incandescent lamps, as was used earlier by Ghaly et al. (2004), for successful horticulture indoors. The 40W lamps were hung in a equidistant pattern 2 feet above the top of the plant canopy and the 100W bulbs were hung further up. The set-up provided 500-600 lux at the top of the canopy. A pho-

Table 1: Composition of the nutrient solution used to acclimatize *H. asiatica* (Garland et al. 1981).

Component	Concentration, mg/L
KCL	150
$MgSO_4$	120
$Ca(NO_3)\ 4H_2O$	946
KH_2PO_4	68
$ZnSO_4.7H_2O$	0.06
H_3BO_3	0.69
$CuCl_2.2H_2O$	0.017
$Na_2MO_4.2H_2O$	0.024
$MnCl_2.4H_2O$	0.022
$FeCl_3$	0.6

Table 2: Pattern of COD removal in SHEFROL®channel containing *H. asiatica* at 3 kg/m² plant density, at HRT 6h, when the influent COD was 600 mg/L.

Number of days from the start of the unit	COD removal (%) in reactors With *H. asiatica*	Without *H. asiatica*
1	57	12
2	63	11
3	65	9
4	67	11
5	71	13
6	74	7
7	72	12
8	70	10
9	74	8
10	72	9
Average ± SD at steady state, i.e. for days 5-10	72.2 ± 1.6	9.8 ± 2.3

Table 3: Pattern of COD removal in SHEFROL® channel containing *H. asiatica* at 3 kg/m² plant density, at HRT 6h, when the influent COD was 900 mg/L.

Number of days from the start of the unit	COD removal (%) in reactors With *H. asiatica*	Without *H. asiatica*
1	57	9
2	65	7
3	71	8
4	70	9
5	78	11
6	84	9
7	85	7
8	88	10
9	86	9
10	84	8
Average ± SD at steady state i.e. for days 6-10	85.4 ± 1.7	8.6 ± 1.1

Table 4: Pattern of COD removal in SHEFROL® channels containing *H. asiatica* at 3 kg/m² plant density, at HRT 6h, when the influent COD was 1200 mg/L.

Number of days from the start of the unit	COD removal (%) in reactors With *H. asiatica*	Without *H. asiatica*
1	47	8
2	55	9
3	61	8
4	73	10
5	75	7
6	77	11
7	85	9
8	83	11
9	86	8
10	87	11
Average ± SD at steady state i.e. for days 6-10	83.6 ± 4	10 ± 1.4

Table 5: Treatment of influent of COD 900 mg/L by *H. asiatica* in SHEFROL® at different HRTs.

Number of days from the start of the unit	COD removal (%) at HRT (hrs) 2	4	6
1	43	53	58
2	45	49	66
3	44	55	69
4	46	59	72
5	55	57	76
6	71	73	79
7	75	78	83
8	78	81	85
9	76	79	86
10	75	82	86
Average ± SD at steady state i.e. for days 7-10	76 ± 1.4	80 ± 1.8	85 ± 1.4

Table 6: Extent of removal of influent COD (1800 mg/L) by *H. asiatica* in SHEFROL® channels at different HRTs.

Number of days from the start of the steady state	COD removal (%) at HRT (hrs) 2	4	6
1	39	45	54
2	40	47	53
3	45	44	45
4	47	48	49
5	41	46	51
6	44	49	50
7	40	43	49
8	44	46	50
9	45	48	51
10	43	47	49
Average ± SD	42.8±2.7	46.3±1.9	50.1±2.5

Table 7: Removal of BOD from sewage containing 600 mg/L of COD and 124 mg/L of BOD by *H. asiatica* in SHEFROL® channels.

Number of days of start of the unit	BOD removal, %
1	71
2	77
3	87
4	91
5	89
6	90
7	92
8	90
9	88
10	91
Average ± SD at steady state i.e. for days 4-10	90.1 ± 1.3

Table 8: Treatment of sewage in terms of nitrogen (total Kjeldahl nitrogen) and phosphorus (soluble phosphorus) removal of *H. asiatica* in SHEFROL® channels at 6 hours HRT.

Number of days of start of the unit	Removal % of Nitrogen*	Removal % of Phosphorus#
1	21	16
2	37	29
3	45	41
4	47	39
5	41	36
6	40	38
7	46	41
8	39	40
9	43	36
10	44	39
Average ± SD at steady state i.e. for days 3-10	43.1 ± 2.9	38.8 ± 2

*Influent concentration, 24.9 mg/L
#Influent concentration, 3.7 mg/L

toperiod of 12 hours was maintained all through by keeping these auxiliary lights on from 7 hours to 19 hours.

Studies outdoors: Outdoor SHEFROL® reactors had dimensions and operational details similar to the ones described for indoor units. As the study area has bright sunshine for most parts of the year, with outdoor temperatures approaching or surpassing 40°C during two-thirds of the time, outdoor units were protected from day-long exposure to direct sunlight, as also from occasional rains that come during November-January or June-July, by an inverted V-shaped roof. The roof was of coconut thatch, supported on a scaffold of sticks obtained from inexpensive *Casuarina* trees. The roof was topped with LDPE sheets for protection from rains.

Analytical quality control: All variables were analysed as per standard methods (APHA 1995). While the precision was ensured by checking the reproducibility, accuracy was affected assessing recovery after standard addition (Abbasi 1998).

Table 9: Treatment of influent of COD 900 mg/L by *H. asiatica* in SHEFROL® channels operated outdoors.

Number of days from the start of the unit	COD removal (%) in reactors With *H. asiatica*	COD removal (%) in reactors Without *H. asiatica*
1	37	9
2	51	6
3	71	7
4	78	10
5	86	9
6	88	8
7	91	11
8	89	8
9	90	9
10	89	7
Average ± SD at steady state i.e. for days 4-10	87.3 ± 4.4	8.9 ± 1.3

Table 10: Removal of suspended solids (SS) contained in sewage by *H. asiatica* in SHEFROL® channels at 6 hours HRT.

Number of days from the start of the reactor	Influent SS concentration, mg/L	Removal of SS, %
1	188	91
2	221	93
3	180	92
4	166	93
5	107	96
6	159	93
7	231	92
8	248	90
9	211	93
10	196	94

RESULTS AND DISCUSSION

In the first set of experiments, the SHEFROL® channels were fed with sewage of COD 600 mg/L at a hydraulic retention time (HRT) of 6 hours. As can be seen from Table 2, *H. asiatica* adapted to the sewage very quickly and was able to remove its COD to the extent of 57% by the end of the first day (24 hours from the start). By the fifth day steady state was reached and thereafter the extent of COD removal averaged 72.2 ± 1.6%. In comparison the channels which had no *H. asiatica* achieved an average COD reduction of only 9.8 ± 2.3%. This much reduction was evidently brought about by the action of light and the atmospheric aeration.

When the reactors were operated with stronger sewage of COD 900 mg/L, the extent of treatment was even better (Table 3). The steady state was achieved as quickly as before and average level of COD removal at steady state was 85.4 ± 1.7%. The control channels achieved only marginal success (Table 3).

The unit was now tested with still stronger sewage of COD 1200 mg/L. It revealed a performance very similar to the one observed in the previous experiment (Table 4).

In the next set of experiments the effect of different HRTs on the treatability of sewage was explored at the influent COD levels of 900 mg/L (Table 5) and 1800 mg/L (Table 6). In both the cases, the extent of treatment followed the order of HRTs: 6> 4> 2, but the difference in the level of treatment was not drastic and even at the very low HRT reflecting exceptionally high throughput of 2 hour, the extent of treatment was only marginally lower than that at the higher HRTs. Considering that the most widely used process for sewage treatment, the activated sludge process and its variants (Abbasi 1999, Abbasi & Abbasi 2018), operate at HRTs of 6 hours or more, SHEFROL® is as efficient, even more.

To assess the extent of BOD, nitrogen and phosphorus removal, their levels were determined in sewage of COD 600 mg/L, before and after treatment in SHEFROL®. BOD was removed to the extent of 90.1 ± 1.3 % at steady state, (Table 7) while the corresponding extents of nitrogen and phosphorus removal were 43.1 ± 2.9 % and 38.8 ± 2% respectively (Table 8).

To assess the ability of *H. asiatica* to affect primary treatment, its capability to handle varying loads of suspended solids (SS) in sewage was tested. As seen from Table 10, the plant was very efficient, achieving 017 ≥ 90% of SS removal.

In studies outdoors, with sewage of 900 mg/L COD, the extent of treatment was 87.3 ± 4.4% (Table 9). It was marginally better than the treatment achieved with the sewage of identical strength indoors % 85.4 ± 1.7% (Table 3). This is as expected because for all plants except the ones who prefer indoor environment, growth would be better outdoor.

The plants remained healthy in all concentrations of sewage and exhibited vigorous growth, particularly outdoors. Despite the reactors having been continuously operated for more than an year, the plants in the reactor channels did not face any pest attack. Only later, in the month of June, a few individuals of green caterpillar, commonly called ‘green cut worms’ (*Pseudaletia unipuncta*), were seen on *H. asiatica* plants, feeding voraciously. They were hand-picked and removed. With it the attack swiftly subsided.

The findings establish the potential of *H. asiatica* to be a very successful bioagent for SHEFROL® in treating sewage. The species was equally successful indoors as well as outdoors, its performance being, as expected, notionally better outdoors given that it is not a particularly shade-specific species. It was able to remove suspended solids, COD and BOD to the extent of 80% and more, indicating that it is able to effect primary as well as secondary treatment very efficiently. There was also substantial tertiary treatment and even though we did not specifically study removal of metals and individual organics, the efficiency of the plant in removing bulk variables (SS, COD, BOD), and in removing N and P, indicates that other pollutants are also likely to be removed.

The ability of *H. asiatica* to treat sewage of strengths varying from 600 mg/L to 1200 mg/L with near-equal efficiency, and to significantly treat sewage of even 1800 mg/L COD, shows that the plant will be able to function well in real-life treatment units where the strength of the influent sewage keeps varying with time. It also reflects the ability of *H. asiatica* to absorb shock loads.

Further, as has been shown by us earlier, when weedy species like *H. asiatica* die in the channels and are removed, or are harvested to check overgrowth, they can be gainfully utilized via anaerobic digestion to obtain energy in the form of flammable CH_4, CO_2 mixture called biogas (Abbasi et al. 1990, Abbasi & Abbasi 2010). Alternatively, or as the next step, spent *H. asiatica* can be converted to organic fertilizer through vermicomposting (Abbasi et al. 2011, Abbasi & Abbasi 2012, Ganesh et al. 2005, Tauseef et al. 2013b, 2014). In this manner the entire *H. asiatica* biomass used in SHEFROL®can be assimilated in nature, leaving nothing to waste.

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