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Short Communication

WASTEWATER TREATMENT USING AQUATIC PLANTS. SURVIVABILITY AND GROWTH OF *SALVINIA MOLESTA* (MITCHELL) OVER WATERS TREATED WITH ZINC(II) AND THE SUBSEQUENT UTILIZATION OF THE HARVESTED WEEDS FOR ENERGY (BIOGAS) PRODUCTION

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Salvinia molesta (Mitchell) is a free-floating aquatic weed, which has colonised several parts of the world, notably Africa [1,2], India [3—5], Australia [6], Ceylon [7], and Papua New Guinea [8,9]. The plant has a very high growth rate [2,10,11] and, in this respect and in terms of adaptability and competitiveness, this weed appears to have an edge over the world's most problematic weed, namely water hyacinth (*Eichhornia crassipes* Mart) [2,12]. Several concerted attempts for the destruction of this weed through chemical methods [13—16], mechanical methods [12,17], and biological methods [4,15,18—20], have been fruitless and attention has recently been turned towards utilization of the weed, so as to compensate for the cost of its mechanical removal. The present study describes one such attempt for the utilization of *Salvinia*. The potential of the weed in treating wastewaters containing zinc(II), and the subsequent conversion of the harvested weed into energy (biogas), have been explored with the end-objective of developing an energy-saving water pollution control system with respect to zinc(II). Zinc was chosen for the study for two reasons. Firstly, it is a common constituent of the wastewaters that are discharged by a large number of industries, viz. pulp and paper manufacturers, viscose rayon producers, electroplating industries, lead and zinc smelters, fertilizer industries, and steam power plants [21]. The second reason was recent findings pertaining to the environmental toxicity of zinc(II) [22,23], which revealed that the prevailing standards for the levels of zinc(II) allowable in industrial wastewater discharges are too high, necessitating further reduction of these levels by appropriate treatment of the zinc-bearing effluents.

EXPERIMENTAL

Filtered well water was used for the growth study. The quality of the test water was determined periodically according to standard methods [24].

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Only slight variation in water quality was observed during the course of the experiment (Table 1). The well water presumably did not contain detectable levels of zinc as the solvent extraction/spectrophotometric and atomic absorption spectrometric methods (sensitivity 0.001 mg/L) failed to detect zinc in the well water.

TABLE 1

Characteristics of the water used in growth study

Parameter		Range
Electrical conductivity	(μS/cm)	27.9–31.7
pH		6.1– 6.3
Alkalinity	(mg/L)	4.0– 6.0
Total hardness	(mg/L)	4.0– 5.0
Calcium hardness	(mg/L)	1.0– 3.0
Sulphate	(mg/L)	Absent (< 1.0)
Chloride	(mg/L)	10.0
Nitrate	(mg/L)	Absent (<0.05)
Nitrite	(mg/L)	Absent (<0.05)
Iron	(mg/L)	0.3
Bicarbonate	(mg/L)	6.0
Total dissolved solids	(mg/L)	87.85
Appearance		Clear

Zinc solution

Stock solutions of zinc(II) were prepared by dissolving reagent-grade zinc sulphate in distilled water.

Studies on survivability and growth of Salvinia

Healthy *Salvinia* plants of free-floating growth stage (plant with small, green, flat leaves) were collected from a pond in Calicut. The plants were washed with distilled water before acclimation.

The standard culture solutions [26] (Table 2) were prepared in 100-L polythene tanks. The pH of the culture solutions was checked by digital pH meter, model IE 1400, and adjusted to 6.0 ± 0.2.

For the acclimation of *Salvinia* plants, seven healthy plants were taken in each trough with 4 L of culture solution. The troughs were 30 cm in diameter, 14 cm in depth, and had a capacity of 5 L. The acclimation period was one week. During acclimation the plants were exposed to sunlight for 6 h every day.

After acclimation the number of plants in each trough was adjusted to five and the number of leaves to forty. The culture solution in the troughs was replaced by freshly prepared culture solution adjusted to pH 6.0 ± 0.2. Zinc

TABLE 2

Nutrient media

Chemical	Concentration (mg/L)
$NaNO_3$	340
$CaCl_2$	166.5
KH_2PO_4	214
$MgSO_4$	514
$FeSO_4 \cdot 7\,H_2O$	5.5
H_3BO_3	0.57
$MnSO_4$	0.57
$ZnSO_4$	0.57

solution was then introduced in the troughs so as to give overall concentrations of 0.0, 1.0, 10.0 and 100.0 ppm. Two controls were kept with respect to which the stimulation (S) or inhibition (I) in growth of *Salvinia* was studied in duplicated experiments.

The plants were allowed to grow in conditions identical to those prevailing during acclimation. The growth was measured each day by counting the number of leaves. The nutrient media were changed fortnightly. The pH was checked daily and was found to be constant at 6.0 ± 0.2.

Utilization of Salvinia *for biogas production*

The experiments were carried out in all-glass airtight digesters of 1-L capacity at 37.5 ± 2.5°C. Each digester was charged with 50 g of *Salvinia* and 375 mL of water. The concentration of zinc(II) (as zinc sulphate) was kept at 0.5 mg (1.18 ppm with respect to total reactants). Five controls were used. The biogas production was noted twice daily. In order to have uniformity in digestion, temperature variations and other environmental conditions, the entire experiment was carried out in one set, with all digesters started at the same time and kept under identical conditions throughout the course of the experiments.

Student's t *test*

The data were analysed in terms of Student's t test to find the significance of zinc(II) on the stimulation (S) or inhibition (I) of growth and biogas production. The t values and confidence levels (CL), obtained from the equations given below, are included in Tables 3 and 4

$$s = \sqrt{\frac{\Sigma(x_1 - \bar{x}_1)^2 + \Sigma(x_2 - \bar{x}_2)^2}{n_1 + n_2 - 2}} \qquad (1)$$

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s} \cdot \sqrt{\frac{n_1 n_2}{n_1 + n_2}} \quad (2)$$

$$v = n_1 + n_2 - 2 \quad (3)$$

Where:

x_1 = observations in the controls;
x_2 = observations in the test sets;
$\bar{x}_1$ = mean of the observations in the controls;
$\bar{x}_2$ = mean of the observations in the test sets;
n_1 = number of the observations of the controls;
n_2 = number of the observations of the test sets;
s = combined standard deviation; and
v = degrees of freedom.

RESULTS AND DISCUSSION

The results are summarised in Table 3. In waters containing 1 ppm and 10 ppm Zn(II), there was a stimulation in the growth of *Salvinia* after a residence time of 10 days. On a few occasions the stimulation was significant at confidence levels of 80—95%. The stimulation was shown throughout the course of the experiment from the 10th day onwards. In water containing 100 ppm zinc, however, the weed could not survive beyond the 15th day, zinc causing highly significant inhibition right from the beginning. The uptake of zinc by the weed was very efficient — 50% zinc being removed within 15 days and 90% within 30 days of growth (Fig. 1).

The survivability and growth of the weed in zinc-contaminated waters, the potency of weed in removing zinc from waters, and the possibility of subsequent conversion of harvested weed into biogas (proceeding section) thus points towards its potential of utilization as a bioagent for the treatment of zinc. It has earlier been demonstrated by Finlayson et al. [6] that *Salvinia* is a very efficient purifier of sewage in facultative wastewater lagoons, removing BOD by 75% and TSS by 7% in excess of the removal achieved in *Salvinia*-free lagoons of identical desing and operation characteristics.

Biogas production from Salvinia

The results are summarised in Table 4. Five sets of controls were used to find out the reproducibility of the biogas yield. The results (Table 4) indicate that the observations are reproducible within ±12% variation. Considering that the digesters contain a heterogenous reactant mass, the reproducibility may be considered as good. The cumulative gas production increases almost linearly with the number of days, with the curve just beginning to taper off after 30 days (Fig. 2). The gas production over the 35-day average works out to be 30.4 L/kg (fresh weight) of *Salvinia*.

TABLE 3

Growth of *Salvinia molesta* over waters treated with zinc(II); dilution water characteristics: cf. Table 1; nutrient media: cf Table 2; pH of experimental water: 6.0±0.2; volume of experimental water in each set: 400 mL; initial number of plants: 5 (40 leaves); period of acclimation: 7 days

Number of days	Control	1 ppm			10 ppm			100 ppm		
	Growth[a]	Growth[a]	*t* value	Stimulation (S) or inhibition (I), at % CL	Growth[a]	*t* value	Stimulation (S) or inhibition (I), at % CL	Growth[a]	*t* value	Stimulation (S) or inhibition (I), at % CL
5	54 ± 16.0	41 ± 0.0	1.0	I[b]	41.5 ± 0.5	0.8	I[b]	11.5 ± 3.5	2.6	I, 80%
10	150 ± 3.0	175 ± 33.0	1.0	S[b]	155.0 ± 8.0	0.6	S[b]	14.0 ± 6.0	20.3	I, 99%
15	332.5 ± 34.5	495 ± 22.0	4.0	S, 90%	423.5 ± 1.5	2.6	S, 80%	16.5 ± 6.5	9.0	I, 98%
20	740.5 ± 69.5	862.5 ± 62.5	1.3	S[b]	813.0 ± 4.0	1.0	S[b]	–	–	–
25	981.5 ± 99.5	1058.5 ± 49.5	0.7	S	1116.5 ± 34.5	1.8	S, 80%	–	–	–
30	1291 ± 55.0	1399.0 ± 59.0	1.3	S[b]	1445.5 ± 115.0	1.2	S[b]	–	–	–
35	1396 ± 140.0	1538.0 ± 100.0	1.0	S[b]	1550.5 ± 85.5	0.9	S[b]	–	–	–
40	1489.5 ± 148.5	1700.5 ± 95.5	1.2	S[b]	1776.0 ± 13.0	1.9	S, 80%	–	–	–
45	1545.0 ± 83.0	1882.0 ± 142.0	2.0	S, 80%	2079.0 ± 4.0	6.4	S, 95%	–	–	–

[a] Average of two sets.
[b] Confidence level less than 80%.

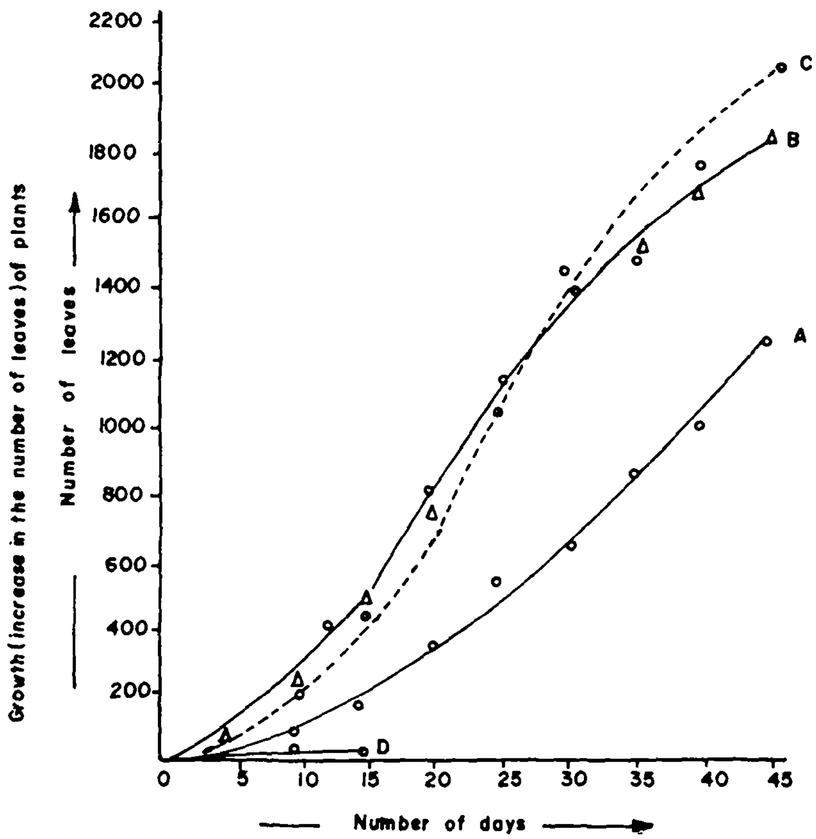

Fig. 1. Average growth of *Salvinia molesta* in waters treated with zinc(II). (A) control; (B) 1.0 ppm Zn(II); (C) 10.0 ppm Zn(II); (D) 100.0 ppm Zn(II).

TABLE 4

Biogas production from *Salvinia* catalyzed by Zinc(II)[a]; *Salvinia*: 50 g; temperature: 37.5±2.5°C; water: 375 mL; zinc(II): 0.5 mg (1.18 ppm)

Sl. No.	No. of Days	Biogas production (mL)					s^b	t^c	Inhibition (I)/stimulation (S)	CL (%)
		Control average	Zinc catalyzed							
			Set A	Set B	Average					
1	5	265.0 ± 33.0	240	325	282.5 ± 42.5		39.1	0.5	S	d
2	10	495.6 ± 77.4	563	628	595.5 ± 32.5		55.3	2.2	S	90
3	15	739.2 ± 79.2	875	972	923.5 ± 48.5		52.3	4.2	S	99
4	20	1039.4 ± 95.6	1251	1057	1154.0 ± 97.0		78.2	1.8	S	80
5	25	1122.6 ± 97.4	1544	1447	1495.5 ± 48.5		91.9	4.1	S	99
6	30	1327.2 ± 124.8	1771	1662	1716.5 ± 54.5		110.7	4.2	S	99
7	35	1523.2 ± 154.8	2108	1922	2015.0 ± 93.0		164.0	3.6	S	98

[a] Methane Percentage was in the range of 50—70%.
[b] cf. eqn. 1.
[c] cf. eqn. 2.
[d] Confidence level below 80%.

The catalytic effect of zinc(II) is shown in Table 4 and Fig. 2. The stimulation is very strong, at confidence levels of 90—99%, right from the start of the digestion till the end. The average gas yield from uncatalyzed *Salvinia* is 30.4 L/kg (fresh weight of *Salvinia*). The 35-day average yield in presence of zinc(II) works out to be 40.3 L/kg (fresh weight) of *Salvinia*. There is thus 33% enhancement in yield in the presence of zinc(II).

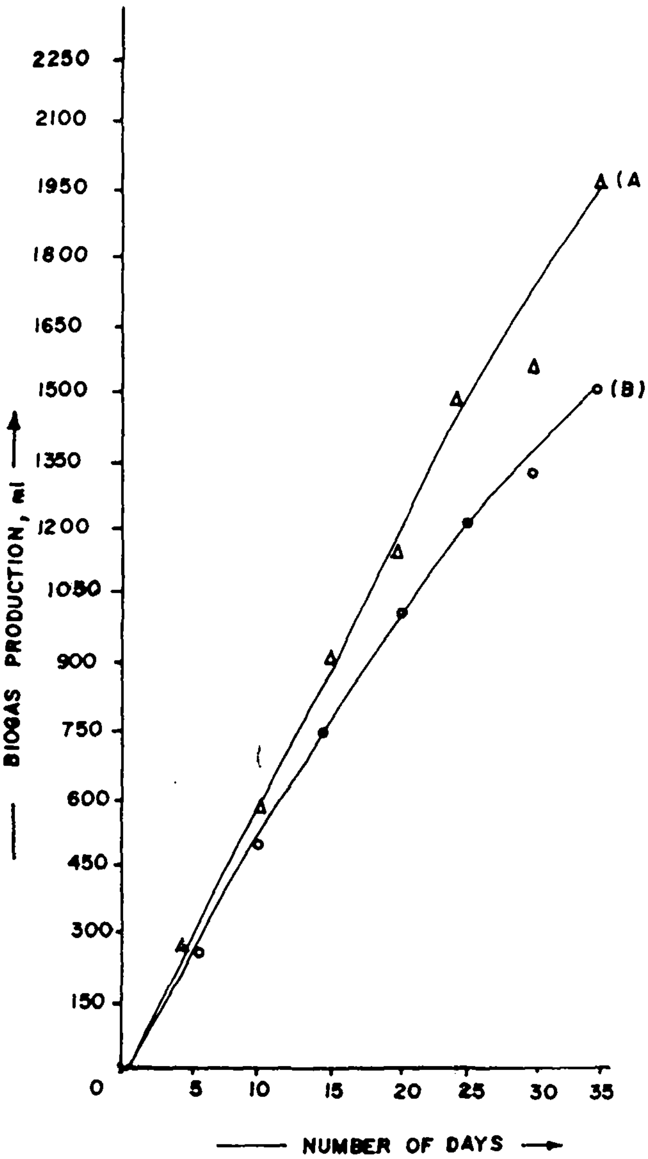

Fig. 2. Average biogas production from *Salvinia molesta* catalyzed by zinc(II). (A) in presence of Zn(II); (B) control.

Several authors [27,28] have earlier reported inhibition in biogas production by zinc and other heavy metals. The common feature of these studies is the employment of zinc in levels exceeding 3000 ppm. At such high levels zinc is expected to be significantly toxic. However, the zinc concentration employed in the present study is at trace level. It appears that zinc causes stimulation in the growth of cellulolytic and methanogenic bacteria at such low levels, and also catalyses some of the biochemical reactions involving electrontransfer. Zinc has been reported to play such roles in other, analogous, situations [29].

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