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Effect of *Eudrilus eugeniae* in vermibioconversion of *Eichhornia crassipes* (Martius) Solms-Laubach

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Abstract

In the present study the physico-chemical status of river Cauvery was analyzed. Rapid additions of nutrients from sewage, dying and industrial effluents were enhancing the growth of aquatic plants like *Eichhornia crassipes* in River Cauvery. The eradication of water hyacinth is tedious due to its fast growth and lack of economically viable options. Hence in the present study an attempt has been made to produce agriculturally important vermicompost from the aquatic weed *E. crassipes* by employing the earthworm *Eudrilus eugeniae.* The nutritional status of *E. crassipes* plant powder (ECP) and earthworm digested *E. crassipes* vermicompost (EWDEV) were analyzed. The cow dung (CD) medium was used as control medium. From the *E. crassipes* the secondary metabolites like alkaloid, flavonoid, steroid, saponin, tannin, glycosides, coumarin, chlorogenic acid anthocyanin, terpenoid and phenol were qualitatively and quantitatively estimated. Among the mediums tested the maximum weight and length was noticed in *E. crassipes* medium than cow dung medium after 50 days. The phytochemicals which are present in *E. crassipes* will not affect the growth or composting potential or nutritional status of vermicompost. The nutrients of *E. crassipes* digested vermicompost were enhanced by the earthworm's digestive enzymes and gut microbes.

Keywords: *Eudrilus eugeniae,* Vermicompost, *Eichhornia crassipes,* phytochemicals.

1. Introduction

Indian ponds, rivers and ground water are used for domestic and agricultural purposes. The quality of water may be described according to their physicochemical and microbiological characteristics. They provide habitat, sanctuary and food for many species of fish and wild life and are also a source of process water to a myriad of industries (Dinar *et al*., 1995). The sources provide water for various activities and in other side these sources out as the sinks for the discharge of domestic as well as industrial waste. It has been observed that major Indian rivers are polluted. The disposal of untreated waste has caused immense problem to both flora and fauna along with aquatic environment worldwide. High degree of pollution by plant nutrients, *viz.* nitrates, nitrites and phosphates has contributed to prolific growth of aquatic plants (water hyacinth), with coverage of 75% of the water bodies. Aquatic plants grow profusely in lakes and waterways all over the world and in recent decades their negative effects have been magnified by man's intensive use of water bodies. Eradication of the weeds has proved almost impossible and even reasonable control is difficult. Turning these weeds to productive use would be desirable if it would partly offset the costs involved in mechanical removal. Among other uses, there has been considerable interest in using aquatic plants as pollution control, especially that the accumulation of heavy metal ions by aquatic macrophytes from the water, in which they were documented by a number of authors (Jamuna and Noorjahan, 2009; Lissy and Madhu, 2010; Valipour *et al*., 2011).

Water hyacinth is the free floating invasive aquatic macrophytes that are known to cause severe damage to the aquatic habitat. The capability of water hyacinth to accumulate plant nutrients and heavy metal contaminants present in water bodies is well known, however, disposal of biomass with accumulated heavy metals is a major constraint. Literature revealed that the noxious weeds like water hyacinth are resisted to the all physical, chemical, biological as well as hybrid methods that have been applied to eradicate it (Abbasi and Ramaswamy, 1999). Large numbers of reports are available regarding utilization of water hyacinth, for example use as paper pulp, poultry/veterinary feed, materials for furniture, carries bags, source of medicinal etc. However, none of them proved to be economically viable options. The only utilization option of water hyacinth that has been found to be economically viable is the treatment of biodegradable waste waters. However, the quantities of water hyacinth used for this purpose are very low and thus disposal problem of the huge waste biomass of water hyacinth is still remaining as a burning problem (Sasidharan *et al*., 2012).

Distribution of *E. crassipes* affects the species composition, abundance, productivity and physiological conditions of aquatic organisms. For aquatic maintenance of water quality through appropriate control measures, continuous monitoring of large number of quality parameters is essential. Besides that the contamination of river water by heavy metals is a serious ecological problem as some of them like Hg and Pb are toxic even at low concentrations, are non-degradable and can bio-accumulate through food chain. Though some metals like Fe, Cu and Zn are essential micronutrients, they can be detrimental to the physiology of the living organisms at higher concentrations (Kar *et al*., 2008; Nair *et al*., 2010). The spatial study of heavy metals by producing heavy metal pollution index can be helpful in identifying and quantifying trends in water quality (Prasadand Kumari, 2008; Reza and Singh, 2010). This may be helpful for resource management and regulatory agencies to make appropriate strategies and necessary decisions. In view of the above, the present investigation emphasis has been given for physico-chemical analysis of river Cauvery and vermibioconversion of waste biomass of *Eichhornia crassipes* by employing *Eudrilus eugeniae*.

2. Materials and Methods

In the present study physico-chemical status of river Cauvery was carried out. The water samples were collected from Cauvery River in 12 to 2.00 pm at the month of July. The samples were collected in sterilized polythene containers completely to exclude any air space, sealed tightly and transported to the laboratory within 24 hours. All the parameters were estimated following the standard method of (APHA, 1998). *Eichhornia crassipes* was collected in the river Cauvery were classified by Dr. M. Palanisamy (Scientist 'C') and voucher specimen (No. BSI/SRC/5/23/2014-15/Tech-669) has deposited in the laboratory of Botanical survey of India, Coimbatore.

E. crassipes were collected from the field and brought to the laboratory and washed with tap water. After removing the moisture the plants were cut into small pieces and dried under shad for 20 days. After 20 days the dried plants were powdered with an electrical blender and sieved to get fine powder. The plant powder is used for earthworm cultivation and a part is extracted with methanol in Soxhlet apparatus for phytochemical analysis. The extracts were subjected to preliminary phytochemicals tests to determine the groups of secondary metabolites present in the plant materials as follows alkaloids, carbohydrate, steroidal glycosides, saponis, tannin, phenol, chlorogenic acid, flavonoids, coumarim, anthocyanin and terpenoid (Harborne, 1998). Based on the preliminary phyotochemical analysis of alkaloid (Harborne, 1973), phenol (Li *et al*., 2008), flavonoid (Ozsoi *et al.*, 2008), steroid (Evans, 1996) and terpenoid were quantitatively estimated. The nutrients of *E. crassipes* powder and its earthworm digested vermicompost were estimated.

The soil used in the experiment was brought from the field and air dried. *E. crassipes*, cow dung and soil (8:1:1%) were amended in containers for pre-decomposition by sprinkling of water for 10 days. After 10 days weighed new baby earth worms (10 No) were introduced into the containers and kept in shade and covered with wire mesh to prevent any rodent attack. Water was sprinkled every day to vermibed for maintain an optimal condition for the earthworms to grow and multiply. The sides and bottom of the containers were perforated to facilitate free aeration and avoid water logging in containers. Three sets of culture media maintained with four sets of replicates with controlled environment. In the present study growth performances of *E. eugeniae* in the form of total growth, weight gain and total length were studied in the medium of *E. crassipes* and cow dung medium.

3. Results and Discussion

Data of the variations of physico-chemical and heavy metal characters of the river Cauvery (July, 2014) are presented in the Table 1. The water sample was appeared to be cloudy white and odourless, while the temperature was 24 ^{0}C temperature. The pH of water and some parameters were comes under the permissible limits. The values of Iron, manganese and Ammonia were slightly higher than the permissible limit. The frequent utilization of nutrients by *E. crassipes* were balance the physico-chemical properties of the River Cauvery. Cauvery River is one of the significant sources of water supply for domestic, agricultural and industrial usage in Tamil Nadu. Inspite of large scale utilization of the river water, poor water management has resulted in large scale degradation of the quality of water. The natural elements which cause water pollution is gases, soil, minerals, humus materials, waste created by animals and other living organisms present in water. Rivers are vital and vulnerable freshwater systems that are critical for the -

Table 1: Physico-chemical and heavy metal analysis of river Cauvery (July-2014)

S. No	Name of the test	Standard values	Cauvery
1	Colour	Colour less	Cloudy White
2	Odour	Odourless	Odourless
3	Appearance	-	Liquid
4	Temperature	-	24
5	pH	6.5 to 8.5	7.8
6	BOD (mg /Lit)	0.3- 1	3.8
7	COD (mg/Lit)	10	150
8	Dissolved oxygen (mg /Lit)	6.0	2.9
9	CO_2 (mg /Lit)	-	0.004
10	Calcium (mg /Lit)	75 - 200	42
11	Magnesium (mg /Lit)	30 -100	82.7
12	Total Hardness(mg /Lit)	300 - 600	500
13	Iron mg /Lit	0.3 -1.5	1.629
14	Manganese mg /Lit	50 - 150	505
15	Ammonia mg /Lit	0.2 - 0.3	1.1
16	Nitrate mg /Lit	45 - 100	78
17	Nitrite mg / lit	1	0.602
18	Chloride ppm	200 - 600	240.18
19	Fluoride mg /Lit	1 - 5	0.201
20	Sulphate mg /Lit	200 - 400	68.74
21	Phosphate mg /Lit	5.0	1.074
22	Sodium mg /Lit	200	173.0
23	Potassium mg /Lit	-	141.0
24	Zinc ppm	5 - 15	0.2942
25	Mercury ppm	0.001	0.0009
26	Lead ppm	0.05	0.00008

sustenance of all life. However, the declining quality of the water in these systems threatens their sustainability and is therefore a cause for concern. Rivers are waterways of strategic importance across the world, providing main water resources for domestic, industrial, and agricultural purposes. The maintenance of healthy aquatic ecosystem is depended on the physico-chemical properties and biological diversity. A regular monitoring of water bodies with required number of parameters with reference to the quality of water not only prevents the outbreak of diseases and occurrence of hazards but checks the water from further deterioration (Venkatesharaju *et al*., 2010).

3.1 Growth of *E. eugeniae* in Different Medium

The different levels of growth of *E. eugeniae* were observed in cow dung and cow dung+ *E. crassipes* mixture mediums. The changes in the worm growth were observed over the period of 50 days were represented in the Table 2. Among the bedding material the maximum weight gain of *E. eugeniae* was observed in *E. crassipes* medium (23.08±0.44 mg/worm) followed by cow dung medium (21.04 ± 0.405 mg/worm) after 50 days. Similar trend was noticed in length of *E. eugeniae* in both the mediums after 50 days. The results from the study clearly indicates that *E. crassipes* with bedding material has positive effect on growth of *E. eugeniae*. The nutrition is as an essential factor to determine the maximum growth of an organism.

The optimal growth, maturation, cocoon production and reproduction potential of earthworms have been depend on quality and quantity of the available feed and various physico-chemical parameters of the culture medium (Karmegam and Daniel, 2000). In the present study the nutritional status of *E. crassipes* were analyzed and are represented in the Table 4. During this study nutrients which are present in the *E. crassipes* promote rapid growth of earthworm and increasing vermicompost production in combination with bedding materials. Composting is techniques often used to reduce water hyacinth into forms utilizable for feeding livestock. Composting is one of the most widely used processing techniques to prepare water hyacinth for use as a fertilizer. A large quantity of inorganic nitrogen and phosphorus accumulates in the roots of water hyacinth, which makes it suitable as a compost or inorganic fertilizer.

The growth rate (mg biomass gained/worm/days) has been considered as a good comparative index to compare the growth of earthworms in different waste or food (Edwards *et al*., 1998). The potential of earthworms as waste processors has been well documented by various authors (Edwards, 1988; Ramalingam, 1997; Suthar, 2007; Sarojini *et al*., 2009). The vermicomposting process refers to earthworm's feed and gut microbial enzymatic activity. During vermicomposting process, inoculated earthworms maintain aerobic condition in the wastes and utilize a portion of organic material into worm biomass (reproduction) and expel the remaining partially stabilized product i.e. vermicompost.

3.2 Nutritional Status of Vermicompost

In the present investigation the maximum pH was noticed in *E. crassipes* and its earthworm digested vermicompost (pH-9) than cow dung (pH-8) medium. The viability in pH could be due to the production of CO_2 and organic acids during decomposition of bedding material. The changes in the pH level may be attribute by the secretion of NH_4+ ions that induce the pool of H^+ ions and the activity of calciferous glands in earthworms containing carbonic anhydrase that catalyses the fixation of CO_2 as $CaCo_3$ thereby preventing the fall of pH (Kale *et al*., 1982).

In this study 0.07, 0.23 and 0.46 mg/gm of nitrogen were noticed in cow dung, *E. crassipes* plant powder and *E. crassipes* earthworm digested vermicompost respectively. From the results, increasing nitrogen level was noticed in *E. crassipes* earthworm digested vermicompost other than cow dung digested vermicompost. The increasing trend of nitrogen level in the vermicompost produced by the earthworm species in the present study corroborated with the findings of earlier studies (Bouche *et al*., 1997; Balamurugan *et al*., 1999). The enhancement of nitrogen in the vermicompost was probably due to the mineralization of the organic matter containing proteins (Bansal and Kapoor, 2000; Kaushik and Garg, 2003) and conversion of ammonium, nitrogen into nitrate (Suthar and Singh, 2008).

The total Phosphorous was higher in the vermicompost harvested at the *E. crassipes* earthworm digested and cow dung mediums than *E. crassipes* powder. Similar results were observed by Kaushik and Garg (2008), Suthar (2007) and Manna *et al*. (2003). The enhanced phosphorus level in the vermicompost suggests phosphorous mineralization during the vermicomposting process (Pattnaik and Vikram Reddy, 2010). The microorganisms present in the worms gut probably convert insoluble potassium into the soluble form by producing microbial enzymes (Kaviraj and Sharma, 2003). During this study the potassium level was increased in *E. crassipes* earthworm digested vermicompost than cow dung digested vermicompost. Sangwan *et al*. (2008) in contrast to the present findings, reported decrease in potassium content in the vermicompost produced by *Eesinia fetida* from industrial sludge.

In the present study the calcium and magnesium level was higher in *E. crassipes* earthworm digested vermicompost than the vermicompost produced from cow dung. The higher calcium activity of carbonic anhydrase present in calciferous glands of earthworms generating $CaCO_3$ on the fixation of CO_2 (Padmavathiamma *et al*., 2008). The higher concentration of magnesium in the vermicompost reported in the present study was also in consistence with the findings of earlier workers (Tiwari *et al*., 1989; Pattnaik and Reddy, 2010a). The higher concentration of magnesium in vermicompost reported in the present study was also in consistence with the findings of the earlier workers (Balamurugan *et al*., 1999; Bouche *et al*., 1997; Morais and Queda, 2003; Manna *et al.*, 2003; Christy and Ramalingam, 2005; Padmavathiamma *et al*., 2008; Sangwan *et al*., 2008; Pattnaik and Reddy, 2010b).

In this study the higher concentration of iron in the vermicompost as compared to cow dung digested compost may be due to mineralization of partially digested worm faecal by detritus communities for example bacteria and fungi (Suthar, 2009). Again, decreased level of Zn in the vermicompost samples may be due to selective absorption of the elements by the earthworm's body tissue. The level of organic carbon was decreased in *E. crassipes* earthworm digested vermicompost (0.06 g/kg) than cow dung medium's (0.012 g/kg) vermicompost. In general organic carbon loss has been observed during the vermicompost process (Suthar, 2007). Earthworm modifies substrate conditions, which consequently affects carbon losses from the substrates through microbial respiration in the form of CO_2 and even through mineralization of organic matter (Suthar and Singh, 2008). Similar kind of results were noticed in vermiconversion of vegetable market waste (MW) and floral waste (FW) processed by three earthworm species namely *Eudrilus eugeniae, Eisenia fetida* and *Perionyx excavates* (Pattnaik and Reddy, 2010a).

3.3 Phytochemical Analysis of *E. crassipes*

In the present investigation the preliminary phytochemical analysis showed the presence of alkaloid, flavonoid, steroid, saponin, tannin, glycosides, chlorogenic acid, terpenoid and phenol (Table 3). Based on the qualitative studies 50.8, 45.2,

Table 2: Weight and length of *E. eugeniae* in cow dung and *E. crassipes* plant powder medium

Number of Day's	**Weight (mg)**		**Length (cm)**	
	Cow dung	***E. crassipes***	**Cow dung**	***E. crassipes***
Initial	14.93 ± 0.16^{f}	14.95 ± 0.19^{f}	0.29 ± 0.14^{f}	f 0.35 ± 0.19^{f}
10 days	17.12 ± 0.08^{e}	15.92 ± 0.14^{e}	5.53 ± 0.08^{e}	5.79 ± 0.14^{e}
20 days	19.08 ± 0.78^{d}	17.16 ± 0.30^{d}	6.38 ± 0.78^{d}	7.00 ± 0.30^{d}
30 days	20.41 ± 0.13^{c}	18.70 ± 0.77^{c}	9.08 ± 0.13^{c}	12.15 ± 0.77^{c}
40 days	22.39 ± 0.72^{b}	20.13 ± 0.18^{b}	11.45 ± 0.72^{b}	15.37 ± 0.18^{b}
50 days	23.08 ± 0.43^{a}	21.05 ± 0.47^{a}	12.95 ± 0.43^{a}	16.06 ± 0.47^{a}
Cd ($p<0.05$)	0.427	0.366	0.364	0.427

Values are mean ± SD (n=10); $^{a-f}$ Mean values with in a column, no common superscript differ significantly at 5% by DMRT

Table 3: Preliminary phytochemical analysis of methanol extract of *E. crassipes*

S. No	**Phytochemical Constituents**	**Name of the Test**	***E. crassipes***
		Mayer's test	+
1	Alkaloid	Dragendroff's test	-
		Wagner Test	+
2	Flavonoids	Ammonia test	+
3	Steroid	Libermann's test	+
4	Saponin	Foam Test	-
5	Tannin	Lead Acetate	+
6	Glycosides	Benedicts Test	-
		Salkowaski test	+
7	Coumarin	Sodium chloride test	-
8	Chlorogenic acid	Ammonia test	+
9	Anthocyanin	H_2So_4 test	-
10	Terpenoid	Borntrager's test	+
11	Phenol	Phenol reagent	+

+ : Present of compound; - : Absent of compound

Table 4: Nutritional status of earthworm digested *E. crassipes* plant powder and its earthworm digested vermicompost

S. No	**Name of the Test**	**Cow dung digested vermicompost**	***E. crassipes* plant powder**	**Earthworm digested *E. crassipes vermicompost***
1	pH	8	9	9
2	Nitrogen mg/ g	0.07	0.23	0.46
3	Phosphorous mg/g	0.26	0.12	0.60
4	Potassium mg/g	71.4	107.1	285.7
5	Calcium mg/g	6	12	14
6	Magnesium mg/g	17.04	19.4	26.7
7	Zinc mg/g	0.19	0.59	0.29
8	Iron mg/g	11.9	20.36	20.2
9	Carbonate and bicarbonate mg/g	0.002	0.004	0.002
10	Chloride mg/g	6.8	10.8	14
11	Organic Carbon g/kg	0.012	0.06	0.06
12	Organic Matter g/kg	0.02	0.15	0.15

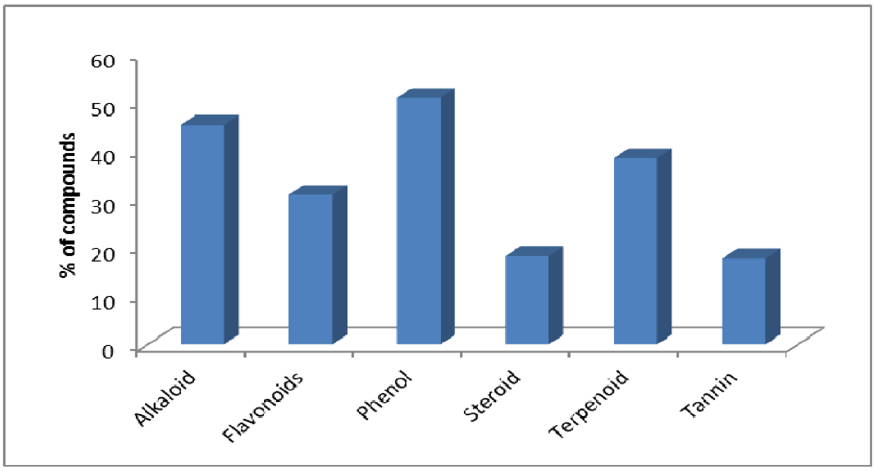

Fig 1: Quantitative phytochemical analysis of methanol extract of *E. crassipes*

38.4, 30.8, 18.2 and 17.7% of phenol, alkaloid, terpenoid, flavonoid, steroid and tannin was noticed in methanol extract of *E. crassipes* (Fig 1). During the study period the phytochemicals of *E. crassipes* were not affecting the biology of *E. eugeniae*. The nutritional status of earthworm digested *E. crassipes* and cow dung were significantly increased due to the earthworms digestive enzymes and gut microbial activity.

Vermicomposting constitutes special forms of composting because it is accomplishes when earthworms metabolize and excrete of soil and organic matter. In the digestive system of worms, micro-organisms are responsible for transferring some organic components particularly protein, nucleic acids, fats and carbohydrates into more stable product i.e. vermicompost. Similarly, in the process of vermicomposting, it supposes that earthworms are useful to clean up the soil from various pollutants, especially heavy metals (Rogars *et al*., 2000; Pereira and Arruda, 2003). Dominguez and Edwards (2004) points out that vermicomposting process involves the active participation of earthworms and microbes. The earthworm homogenizes the ingested material through muscular action of its foregut, adds mucus and provides enzyme rich environment to the material taken up by the worms, thereby increasing the surface area for microbial action, while the microorganisms perform the biochemical degradation. The microbial action would be complete in the extracellular enzymatic environment of earthworms.

4. Conclusion

Now a days the water hyacinth becoming a major problem in water resources that through all over the world. The plant absorbs excess nutrients from the water resource and shows the excess growth. From the *E. crassipes* the stored nutrients were converted into economically important vermicompost by employing the earthworm *E. eugeniae*. This result concluded that the enrichment of vermicompost by addition of *E. crassipes* plant is used as a substrated medium.

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