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Enrichment of Physico-Chemical Parameters of Sludge Using Earthworm *Perionyx Ceylanesis*

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Abstract:

Wastewater treatment processes produces by-product in the form of sludge which can be used as fertilizer for the soil and crops. The application of treated sludge in the form of fertilizer can significantly reduce the disposal cost of sludge as well as provides large part of macro and micro nutrients for the plants and crops. The treated sludge can be used for providing substantial amount of required nitrogen and phosphorus for different crops.

The application of a new species of earthworm on the sludge has improved the quality of various parameters present in the sludge. The content of both macro and micro nutrients present in the resultant compost has been increased which can be used as a good of fertilizer.

The earthworm species *Perionyx ceylanesis* named as "Jai Gopal" has various characters namely high fecundity, heat tolerance up to 43^0C ambient temperature, rich protein content of 67% and almost all functional amino-acids. It has longer life span, the interval of time period from hatchling to maturity is smaller and the adoption ability from 23 to 43^0C ambient temperature makes is more efficient, feasible and durable.

The results of the present study have clearly explained that all the parameters related to nutrient supply and fertility (e.g. organic carbon, nitrogen, phosphorus, potassium, zinc, iron, magnesium and sulphur) have increased significantly, while pH and electrical conductivity have decreased considerably. Thus it can be concluded that vermicompost prepared from *Perionyx ceylanesis* named as "Jai Gopal" has significantly increased the nutritive status of the treated soil.

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I. Introduction

The food requirements of present generation are of the most important demand of sustainable agriculture in the present scenario. The adverse impact of agrochemicals on our land, water, food and overall environment had reaching alarming level [1-4]. We have degraded our environment through unsustainable agricultural practices. Thus to avoid further harm to environment and to move towards sustainable agriculture, organic farming is the best option. Vermicomposting uses earthworm for the preparation of compost. It not

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only provides vital nutrients necessary for plant growth but also helps in maintaining human health by avoiding toxic contamination in soil, food grains and water [5-9]. Vermicomposting or processing of sludge into useful manure is carried out by different species of Earthworm. One of the most frequently used species for vermicomposting is *Eisenia fetida*. Recently a new variety of earthworm was developed by Indian veterinary Research Institute (IVRI), Izzatnagar, Bareilly known as Jai Gopal (*Perionyx ceylanesis*) which is developed by mating and selection of *Eisenia foetida* with *Eudrilus eugeneae*. Its advantages include high fecundity, high heat tolerance up to 43 ^{0}C, small time period from hatching to maturity and they are having long life span with voracious feeder. Addition of this compost in soil improves its physical, chemical and biological characteristics as well as helps in growth of plants without harming environment (Table 1) [10-12].

II. Materials and Methods

Study area location and climatic conditions:

Mathura is located in the Indian state of Uttar Pradesh. It is located about 145 kilometres (90 mi) south-east of New Delhi. It is located at 27.28°N 77.41°E and an average elevation of 174 metres. The climate is tropical extreme with very hot summers with temperature rising to 44 °C, and extreme cold winters with temperature dropping to 5 °C. The average rainfall recorded is 793 mm which is mostly received during the month from July to September.

Vermicomposting Formation:-

Vermicomposting pit of size 1.5m × 2m×1.5m was prepared for the treating of sludge obtained from sewage treatment plant. Then the pit was filled with cow dung and sludge. Cow dung was used as composting material. Cow dung and sludge was mixed in the proportion of 1:1 and is allowed to partially decompose for 15 days. 1500-2000 Earthworms were inoculated in each pit (Fig 1).

Proper aeration should be maintained in vermicomposting unit by regular turning the composting material. Optimum moisture level (30 to 40%) is maintained by regular sprinkling of water. Vermicomposting pit is kept in cool, shady place to maintain optimum temperature around 18 to 25°c for proper decomposition (Fig 2) .

Fig: 1 Vermicomposting of sludge with cow dung by species Jai Gopal (*Perionyx ceylanesis*)

Fig: 2 Jai Gopal (Perionyx ceylanesis) Earthworm Species in a mixture of Sludge and Dung

Sludge is converted into compost by two species Red worms (*Eisenia fetida*) And Jai Gopal" (*Perionyx ceylanesis*) (Table 1)

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S. No.	Name of earthworm	Characteristic
1	"Red worms" (*Eisenia fetida*)	Small, red in colour, tolerates wide range of environmental conditions, can feed on wide variety of organic wastes, faster growth and Multiplication.
2	"Jai Gopal" (*Perionyx ceylanesis*)	Developed through selection and mating plan which is better than exotic earthworm *Eisenia fetida, Eudrilus eugeneae*

Table-1 Characteristics of Earthworm Species

Study on Physicochemical Parameters-

Soil *p*H-

For the determination of soil pH, calomel electrode method was used [13]. In this method soil and distil water were mixed in the ratio of 1:5 (w/v) and vertex for at least 30 min. After proper mixing the pH of the soil suspension was measured with the help of pH meter.

Electrical conductivity-

Electrical conductivity may be defined as the capacity of water to allow electric current. In simple words electrical conductivity is directly proportional to the dissolved minerals content. Soil was mixed with distil water in the ratio of 1:5 (w/v) and the mixture was vortex for 30 min. Then the electrical conductivity of the soil suspension was measured by conductivity meter.

Soil organic carbon-

Organic carbon in soil was estimated by using the method of Piper (1966). Weigh 1-2 g air dried soil in an Erlenmeyer flask of 500 ml capacity and to this 10ml of N K2Cr2O7 and 20ml of H2SO4 were added in succession. The suspension was shaken for 1 min by hand and placed for 30 min on an asbestos sheet. After this added 200ml distilled water, 10ml phosphoric acid and 1ml diphenylamine indicator solution into it. This suspension was titrated with N FeSO4.7H2O until the solution turned blue. Continue adding ferrous sulphate drop by drop until greenish color occurred that was considered as the end point. The percent of organic carbon was calculated by using the following formula:

$$\text{Organic carbon}(\%) = \frac{(V1 - V2)\ \text{X}\ 0.003}{W}\ \text{X}100$$

V1 = Volume of N $K_2Cr_2O_7$, V2 = Volume of N FeSO4 7H2O, W = Weight of soil taken

Total nitrogen -

Semi micro-Kjeldahl method was used for the estimation of total nitrogen as suggested by some researchers [14-15]. 10 g soil sample was taken in a Kjeldahl digestion flask and to it 5ml of water was added. 15 ml concentrated H2SO4 was added with constant shaking. To this, small quantity of KMnO4 was added till pink color developed. Now add catalyst mixture (3g K2SO4 + 0.3g FeSO4.7H2O + 0.15g CuSO4.5H2O) to it. This mixture was digested for 30 min at low temperature till yellow-green color appeared. After proper cooling this mixture was transferred to 100ml volumetric flask though repeated washing with distil water and make the final volume with 100ml.

Now steam distillation was performed by adding 30ml of 40% NaOH into 20ml of the digested solution in the funnel of distillation apparatus. At the same time a beaker was taken and 10ml of N/10 H2SO4 was added along with 3-4 drops of methyl red indicator. This beaker was put under the delivery tube of the condenser in such a way that the tip of the condenser positioned below the liquid surface. The contents of the distillation flask were boiled by passing the steam. The distillate was collected and titrated with N/10 NaOH till pink color of methyl red turned yellow after neutralization.

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Calculation of total nitrogen was done by considering the fact that 1ml N/10 H_2SO_4 = 0.0014g nitrogen and expressed in terms of percent.

Phosphorous-

Take a plastic bottle of 100ml capacity and 10g of soil was added to it. Transfer 50 ml of extracting solution to it and shake it for 60 min. Filter this solution through a Whatman No. 42 to get the filtrate. The blank was included. Now, blank and extract (4 ml) were taken into a boiling tube and to this 1ml distil water and 1ml of molybdate - vanadate were added. The mixture was allowed to stand for 20 min and OD was measured by using UV/Visible spectrophotometer.

Exchangeable potassium -

1g soil was taken into a conical flask and to it 25 ml of tri acid solution (H_2SO_4: HNO_3: $HClO_4$ = 9:3:1) was added to form a suspension. The suspension was heated constantly till it becomes transparent. With the help of distil water, make the total volume upto 100 ml. The solution was filtered by using Whatman filter No. 50. Potassium concentration was calculated by using flame photometer.

Zinc -

For the estimation of zinc in soil sample, a stock solution of Zn (100 mg/l) was prepared by dissolving 0.4398 g of ZnSO4.7H2O in distilled water and diluted to 1000ml. This stock was diluted to obtain working standards of range of 0 – 5 mg/l. Now the sample solution and standards were aspirated in the flame. The concentration of zinc can be calculated by using the calibration curve.

$$\text{Conc. of Zinc (mg/kg)} = \frac{(a-b)\text{ x V x f x } 1000}{1000\text{ x w}}$$

Where: a- concentration of Zn in the sample extract, b- concentration of element in the blank extract, v- volume of the extract solution, w- weight of the soil sample and f- dilution factor.

Iron-

Prepared a standard stock solution of Fe (100 mg/l) by adding 0.1 g of clean untarnished iron in 10 ml of warm 10% H_2SO_4. After attaining the room temperature, make the final volume upto 1000 ml. From this stock solution different working concentration 0 - 20 mg/l were made. The concentration of iron was calculated by atomic absorption spectroscopy at operating wavelength. The concentration of iron in the sample can be calculated from the calibration graph.

Exchangeable magnesium-

7.5 g of air dried soil was taken in 500ml beaker and 15 ml of 40% alcohol was mixed in it. The mixture was shaken gently for 15 min and filtered through Whatman No-50 filter paper. Wash the sample with 7.5 ml of 40% alcohol solution for 4-5 times. Final washing was done by 7.5 ml of absolute alcohol. Soil was scrapped from the filter paper in a 250 ml beaker and washed with ammonium acetate solution for eliminating any adhered soil particle. Keep the solution overnight. Now filter the solution by using Whatman No. 42 filter paper by adding more ammonium acetate solution 50ml volumetric flask. To this filtrate 2 ml NaOH and 200 mg murexide indicator ware added. Pink color developed. Now titrate this solution against EDTA until pink color transform into purple. Magnesium concentration was determined by using following formula-

$$\text{Conc. of Mg (\%)} = \frac{(B-A)\text{ x } 400.8\text{ x V}}{\text{v x } 1.645\text{ x } 1000\text{ x S}}$$

Where A- Volume of EDTA used for calcium determination, B- Volume of EDTA used for calcium + magnesium determination, V- Total volume of soil extract prepared, S- Weight of soil taken, v = Volume of soil extract titrated.

Sulphur-

20 g soil sample was taken into 250 ml conical flask. To this 100 ml of the monocalcium phosphate extracting solution (500 mg/1) was

added and shake the suspension for one hour. Filter this suspension with Whatman No. 42 filter Paper. Now take 10 ml filtrate into a 25 ml volumetric flask. To this 2.5 ml of 25% HNO_3 and 2 ml of acetic-phosphoric acid were added. Seed suspension of $BaSO_4$ was gently shaken and 0.5 ml of it is added into 0.2 g of $BaCl_2$ crystals. Allow the suspension for about 5-10 min. Now add 1 ml of gum acacia-acetic acid solution was added to it and invert the solution for 10 times. Now measure the OD at 440 nm in spectrophotometer.

$$\text{Available sulphur (mg/kg)} = \frac{\text{R x 100}}{\text{10 x 20}}$$

Where, r stands for the quantity of S in mg as obtained on X-axis against a reading.

III. Result & Discussion-

The analysis of sludge and vermicompost formed by 'Jai Gopal' Earthworm species using STP sludge and cow dung in 1:2 ratio indicates that resultant vermicompost is superior in quality in comparison to the sludge from wastewater treatment as it is having higher percentage of most of the important soil parameters like organic carbon, electrical conductivity, total carbon, nitrogen, phosphorus, potassium, zinc and iron.

The results of the present study clearly indicates that there is decline in the pH and electrical conductivity as we applied the vermicompost prepared from earthworm 'Jaigopal' (*Perionyx ceylanesis*), that is a major sign of improvement in the soil salinity and soil fertility. Similar findings were obtained by other workers (Fig 3 and 4) [16-18].

Vermicompost prepared from Jaigopal (*Perionyx ceylanesis*) is having higher proportion of organic carbon (53.2%) as compared to sludge (52.6%) with percent increament of +11.40%, So it will enhance soil fertility more by improving structure of soil and number of beneficial microorganisms (Table-2). Physical properties of soil like waterholding capacity, infiltration, gaseous exchange and root growth will also be promoted. Increased presence of nitrogen (32.3 mg/kg from 31.26 mg/kg) in vermicompost will affect carbohydrate and protein synthesis, photosynthesis, increase in leaf surface (shoot growth), length of growing season, and delays maturity. Several research papers also depicted the similar findings (Fig 4) [16, 19-21].

Vermicompost with higher percentage of phosphorus (0.63% from 0.62%) is likely to have early root and shoot development and better formation of seeds and fruit. Increased level of potassium (17.2 mg/kg from 16.88 mg/kg) will increase photosynthetic activity of leaves, synthesis of amino acids, proteins and sugar, resistance to disease is increased along with increasing plumpness of the grains. From the data obtained from this study it has become clear that there is 1.61% and 1.89% increase in the phosphorous and potassium content respectively (Table-2) (Fig 4). Other workers have also obtained the similar results [22-24].

From Table-2 it has been depicted that enhanced concentration of zinc (2.6 mg/kg from 2.4 mg/kg) will help the plants in increased growth, seed and bud development. In case of some other parameters such as Fe, Mg and S it has been clear that vermicompost prepared from *Perionyx ceylanesis* species is very rich in proteins and there is presence of all functional amino acids, that's why all these content have increased from the untreated sludge. There was an increase of 23.80%, 9.95% and 1.72% in the concentration of Fe, Mg and S respectively. These results were also show the similar pattern of increase in the nutrient concentration (Fig 4) [22, 23, 25]. So, we can say that the compost prepared from Jaigopal (*Perionyx ceylanesis*) is having better in physical and chemical characteristics in

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S. No.	Item	pH (1:5 Suspension)	EC (µS)	OC (% by mass)	N (mg/ kg)	P (% by mass)	K (mg/ kg)	Zn (mg/ kg)	Fe (mg/ kg)	Mg (% by mass)	S (mg/ kg)
1	STP Sludge	7.24	2260	52.6	31.26	0.62	16.88	2.4	0.42	4.82	52.2
2	Enriched Sludge	7.1	1987	53.2	32.3	0.63	17.2	2.6	0.52	5.3	53.1
3	% Change	-1.97	-12.07	+11.40	+3.32	+1.61	+1.89	+8.3	+23.80	+9.95	+1.72

Table-2: Comparison of STP Sludge and Sludge formed by Jai Gopal Earthworm using STP Sludge and cow dung in 1:2 ratio.

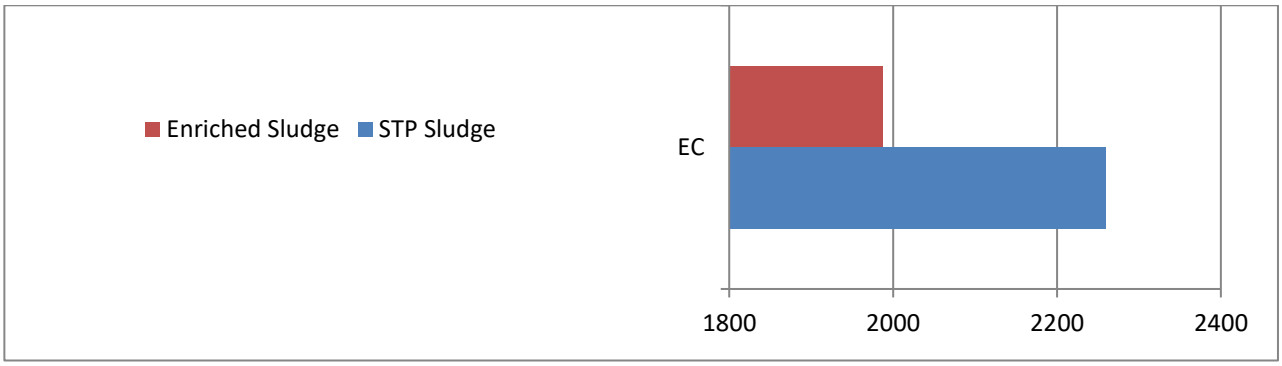

Figure 3- Comparison of electrical conductivity in STP sludge and vermicompost enriched sludge.

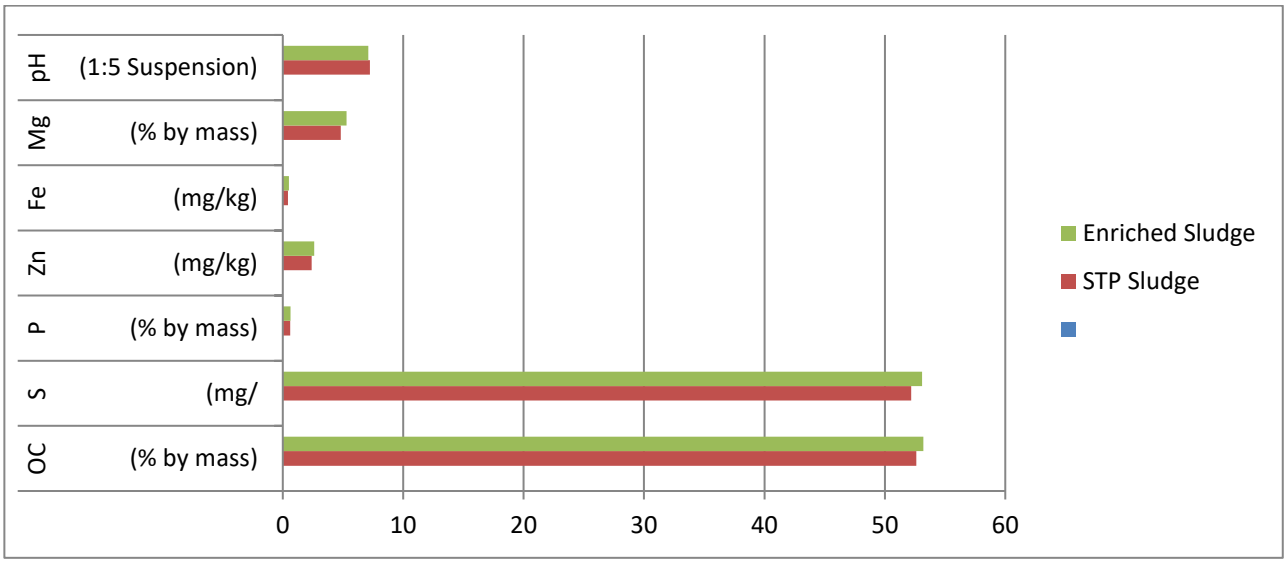

Figure 4- Comparison of Micro and Macro elements in STP sludge and vermicompost enriched sludge.

comparison to sludge and is likely to have better impact on plant growth if we utilized it as a growing medium.

IV. Conclusions

The application of new earthworm species 'Jaigopal (*Perionyx ceylanesis*)' on the sludge has improved the physical and chemical characteristics of the sludge. The nutrient quality of the sludge has increased which can be used as a fertilizer for various agricultural practices. Chemical fertilizers although help in food production but they are harmful for environment in the long run. The advantage of using enriched sludge as a medium for plant growth is that it improves soil fertility without harming environment especially soil. The nutrient quality of the soil is improved and the toxicity of soil due to use chemical fertilizers is removed, so overall agriculture is sustainable.

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