Earthworms in the second decade of 21st century are the most significant figure in the realm of biodegradation of agricultural wastes, agro-industrial wastes, urban organic garbage and sewage sludge. The degraded product as their excreta find resolvable route to sustainable agriculture, a most relevant solution for the developing country like India to enhance sustainability of soil organic matter for balance of physical-chemical biological properties. Earthworms virtually stand alone as a symbol of “clean technology” in the field of organic waste transformations and also stand alone as a symbol of “sustainable organic agriculture technology (Seema et al.2013).

**Standardization of the Culture Techniques**

The efficiency of vermicomposting process using (*E. fetida* epigeic species) on the basis of nutrient content (N, P) of the compost was found maximum for industrial waste followed by institutional waste, agroresidues and kitchen waste (Garg *et al.* 2006). The percentage of, nitrogen, phosphorous and potassium in vermicompost was found to increase while pH and total organic carbon declined as a function of the vermicomposting period. 4.4–5.8-fold increases in TKN (Total Kjeldahl Nitrogen) was observed in different feed mixtures at the end of vermicomposting period. The increase in TKN for different feed substrates was found in the order: textile sludge > textile fibre = institutional waste > agro-residues > kitchen waste. Available Phosphorus increased 1.4 to 6.5-fold in different feed mixtures in comparison to control. Reduction in total organic carbon was highest in agro-residues (3-fold) followed by kitchen waste (2.2-fold), institutional waste (1.7-fold) and textile industrial wastes (sludge: 1.5-fold and fibre: 1.68-fold) in earthworm-inoculated pots than control. The data reveals that vermicomposting (using *E. fetida*) is a suitable technology for the decomposition of different types of organic wastes (domestic as well as industrial) into value-added material.

Vermicomposting resulted in significant reduction in C:N ratio and increase in TKN. Total K and Ca were lower in the final cast than the initial feed mixture. Microbial activity measured as dehydrogenase activity increased up to 75 days and
decreased on further incubation. Total P was higher in the final product than the initial feed mixture. Total heavy metal contents were lower in the final product than initial feed mixture (Kaushik and Garg, 2003).

Experiment showed that cellulose substrate was the best for earthworm culture and had the highest total weight gain, relative growth rate, specific growth rate, feed conversion ratio and biomass production of 307.9 g, 332.15%, 0.76%, 1.43 and 25.7 g/week, while the control substrate (soil) recorded the least values of 213.6 g, 230.4 %, 0.62 %, 1.76 and 17.8 g/week respectively (Sogbesan et al. 2006).

Vermicomposting resulted in significant increase in total N (80.8–142.3%), phosphorous (33.1–114.6%) and potassium (26.3–125.2%), whereas decrease in organic C (14.0–37.0%) as well as C:N ratio (52.4–69.8%) in different experimental beddings. *P. sansibaricus* showed maximum biomass production, growth rate (mg/day), mean cocoon numbers, and reproduction rate (cocoon/worm) in VLL (vegetable waste + leaf litter) as compared to other substrate materials. There was a consistent trend for earthworm growth and reproduction rate, related to initial N-content of the substrate (P < 0.05), but there was no clear effect of C:N ratio of the composted material on earthworm cocoon numbers and weight gain. Earthworms showed minimum total population mortality in VLL and maximum in HHCD (household waste + cow dung), after 150 days of experimentation. The increased level of plant metabolites in end product (vermicompost) and growth patterns of *P. sansibaricus* in different organic waste resources demonstrated the candidature of this species for wastes recycle operations at low-input basis (Suthar, 2006).

The cocoon production per worm in cattle manure was higher than in goat manure. However, the hatchability of cocoons was not affected by manure treatments. Cattle manure provided a more nutritious and friendly environment to the earthworms than goat manure (Lee, 2003).

The vermicomposting caused a decrease in organic C (12.7–28%) and C:N ratio (42.4–57.8%), while increase in total N (50.6–75.8%), available P (42.5–110.4%), and exchangeable K (36.0–78.4%) contents. Waste mineralization and humification rates were higher in bedding those containing easy digestible bulky
agents, i.e., BGS (Biogas Slurry) and CD (Cow Dung). Worm-processed material obtained from BGS:VW (1:2) vermibed showed the higher total N (31.3 g kg\(^{-1}\)), available P (8.7 g kg\(^{-1}\)) and exchangeable K (20.7 g kg\(^{-1}\)) contents. The nutrient-rich vermicompost with acceptable C:N ratio ranges (≥1:20) indicates its agronomic potentials. Waste mixtures also supported the earthworm growth and reproduction rates in vermibeds (Suthar, 2009). Earthworm mortality in vermibed could be related to the quality as well as chemical composition of the substrate used for earthworm culture.

### The Species and Organic Waste Specific Nutrient Profile of Vermicompost

Vermiculture technology is more efficient, economically viable, environmentally positive, practically feasible recycling process and involves the use of earthworms as versatile natural bioreactors for effective recycling of non-toxic organic solid and liquid wastes in the soil. Vermicomposting is the process by which worms are used to convert organic materials (usually wastes) into a humus-like material known as vermicompost. Vermicomposting helps in productive utilization of organic wastes materials as agricultural wastes, animal dropping, forest litter and agro-based industrial wastes. Vermicompost improves the physical, chemical and biological properties of the soil and better crop productivity. Earthworms effectively harness the beneficial soil micro flora, destroy soil pathogen and convert organic wastes into vitamins, enzymes, antibiotics, plant growth hormone, protein rich products and others organic compounds. Vermicompost is becoming an impartment alternative to conventional compost and FYM sources for organic farming. It also controls soil, maintain the soil health as well as environmental pollution.

A greater proportion (>80%) of biomass of terrestrial invertebrates is represented by earthworms which play an important role in structuring and increasing the nutrient content of the soil. Therefore, they can be suitable bioindicators of chemical contamination particularly heavy metals of the soil in terrestrial ecosystems providing an early warning of deterioration in soil quality. The suitability of earthworms as bioindicators in soil toxicity is largely due to the fact that they ingest
large quantity of the decomposed litter, manure, and other organic matter deposited on soil, helping to convert it into rich topsoil. Earthworm skin is a significant route of contaminant uptake and thus investigation of earthworm biomarkers in the ecological risk assessment (ERA) can be helpful. Growth is an integral parameter easy to measure, and it integrates a suite of biochemical and physiological effects. It represents changes in individual energy budgets as the exposed organisms have to expand their energy to metabolism, detoxification or sequestration, and excretion of the contaminants. This additional energy requirement results in a decrease of growth. Furthermore, the earthworm reproduction test is widely regarded to be of greater significance for predicting the impacts on soil ecosystems. Reproduction is very important in ecotoxicological assessment as it influences the population dynamics. Reproduction rate was shown to be a sensitive endpoint in toxicity tests of various metals and organic compounds. It can be used for the management of weeds and wastes from agriculture, industries, kitchen etc. Research on vermicomposting of a variety of wastes is gaining momentum throughout the world (Frederickson et al., 1997; Edwards, 1988; Kale, 1998; Sinha et al., 2002).

**Effect of Heavy Metal on the Growth, Survival and Reproductive Potential of Different Species.**

A number of studies have shown that species richness and diversity of earthworms decreased along a gradient of metal pollution (Pižl and Josens, 1995; Lukkari et al., 2004), and most laboratory ecotoxicity tests or field studies have shown negative effects of metals on survival, growth, feeding activity and reproduction of earthworms (Gupta et al., 1997, Edward and Lofty, 1982).

The production of cocoon starts at the age of 6 weeks and continues till the end of 6 months. Under favorable conditions one pair of earthworms can produce 100 cocoons in 6 weeks to 6 months (Ismail, 1997). The incubation period of a cocoon is roughly about 3-5 weeks however in temperate worms it ranges between 3-30 weeks and in the tropical worms between 1-8 weeks. Earthworms also have the power to regenerate segments, which are lost. The doubling time i.e. the time taken by a given earthworm population to double its number or biomass, specifically depends upon the earthworm species, type of food, climatic condition etc. For example, the mean
doubling time, with reference to density and biomass of *Lampito mauritii* in different organic inputs is 38.05 and 33.77 days respectively while in case of *Perionyx excavatus* it is 11.72 and 16.14 days respectively (Ismail, 1997). The most effective use of earthworms in organic waste management requires a detailed understanding of the biology of all potentially useful species (Edward, 1988). Reinecke et al. (1992) reported that *E. fetida* had a wider tolerance for temperature than *Eudrilus eugeniae* and *P. excavatus*. It tolerates as high as 42°C as well as low soil temperature below 5°C. Such influence of environmental factors specially temperature on the fecundity of vermicomposting earthworms are on record (Venter and Reinecke, 1988; Giraddi, 2000). Besides, effect of weather parameters such as profound influence of temperature on growth and development of worms (Reinecke et al. 1992) bed moisture and the types of food substrate (Venter and Reinecke, 1988) also determine the hatching success.

The incubation period which is slightly longer in *P. excavatus* varied from 20 to 21.7 days, but in *E. eugeniae* it ranged from 15.3 to 16.8 days. Variation observed in the incubation period of the two species during present study periods was found to be non-significant. The hatching percentage and the number of neonates/cocoon and hatching potential of cocoons that indicate the cocoon viability, was in general higher (74.5 to 91.5%). The hatching percentage in both the species was significantly higher in rainy and winter a month which was characterized by a lower temperature i.e. 20 to 24°C (Giraddi, 2000). Studies on the comparative reproductive biology of vermicomposting earthworms were taken up at the Main Agricultural Research Station, UAS, Dharwad during 2004-05, so as to assess the reproductive potential across the different seasons. *Eudrilus eugeniae* (Kinberg) was observed to have mean fecundity of 6.75 cocoons/week (with a range of 5.4 during summer months to 7.75 during rainy season) as against 2.63 cocoons/week (2.10 during summer to 3.00 in rainy months) seen in *Perionyx excavatus* (Perrier). The incubation period was 16.2 days in *E. eugeniae* as compared to slightly higher duration (20.7 days) in *P. excavatus*. Number of neonates per cocoon was higher (2.71) in *E. eugeniae*, where as it was 0.81 in *P. excavatus*. Seasonal variation appeared to exert no influence on the incubation period as well as neonate numbers /cocoon, for both the species. The
fecundity however reduced in summer. Hatching percentage which speaks on the viability of cocoons was higher (85.3) in *E. eugeniae* than 68.3% observed in *P. excavatus* and was influenced by seasonal changes in both the worms. The results obtained in the study indicated that *E. eugeniae* is the prolific breeder as compared to *P. excavatus.* (Giraddi *et al.* 2008)

In order to assess the response of epigeic earthworms to seasonal changes population dynamics of *Eisenia fetida* (Oligochaeta, Lumbricidae) was monitored in a manure heap in the field during a year. Earthworms were hand-sorted from five 0.25 × 0.25 × 0.20 m blocks around the heap in November (autumn) 1999 and in January (winter), April (spring) and August (summer) 2000, so as to determine the earthworm population dynamics. Earthworms of each block were classified into different age classes: i.e. mature, preclitellate, juvenile, hatchling and cocoon, and afterwards counted and weighed. Seasonality had a strong effect on the density, biomass and the reproductive activity of the population. Population of *E. fetida* was characterized by a high density of individuals and the predominance of mature individuals throughout the year. Maximum density, mating activity and the size of cocoons were achieved in spring, but there were no change in the number of cocoons per mature earthworm throughout the year. Unexpectedly, the smallest cocoons were produced in winter by the largest individuals. These results suggest that *E. fetida* is able to allocate resources to growth and/or reproduction in response to environmental fluctuations (Monroy *et al.* 2006). A maximum mean weight of adult earthworms was achieved in winter at the lowest temperature. But these large-sized earthworms showed the lowest mating activity (Kaplan *et al.* 1980). Temperature regimes below 15°C cause low growth rates and also affect the reproduction thereby diminishing the cocoon production and increasing incubation time. The low reproductive activity found in winter could lead to weight gain due to decrease of reproductive costs and reallocation of resources toward growth. According to this, the mean weight of the mature earthworms decreased in spring with the increasing reproductive activity (Stearns, 1992). *E. fetida* can however reach nearly 1.6 g in laboratory conditions. (Venter and Reinecke, 1988).
Edwards et. al. (1998) studied the growth patterns of *P. excavatus* on separated cattle solids at four different temperatures: 15, 20, 25, and 30 °C. They reported the range of growth rate between 0.6 (mg/worm /day) and 7.6 (mg/worm/day) at 25–30°C. The difference in growth rate observed in that of earlier work might be due to fluctuating temperature or due to difference in substrate quality. The maximum biomass increase in different beddings differed from those of the earlier reports (Reinecke et al., 1992; Edwards et al., 1998).

The difference for cocoon production rate in present and past studies could be related to the quality of the substrate. Edwards et al. (1998) concluded that the important difference between the rates of cocoon production in the two organic wastes must be related to the quality of the waste material. The maximum mass gain, growth rate, and cocoon production and earliest attainment of maturity were recorded during the monsoon season, and were attributed to the lowest range of fluctuating temperatures and high humidity as compared with observation made during winter and summer. The growth rate of worms in all seasons was inversely proportional to the number of cocoons produced. Further it has been reported that the higher the rate of cocoon production, lower is the weight of individual cocoons (Biradar et al., 1999).

The maximum overall productivity probably depended upon the total biomass and the nutritive value of microorganisms growing on the waste (Edwards and Fletcher, 1988). The growth and reproduction of *Eudrilus eugeniae* (Kinberg) in cattle waste solids was studied by growing groups of 1, 2, 4, 8 or 16 small earthworms in 100 g of waste in small containers in incubators at 15°, 20°, 25° and 30°C. Earthworms were weighed weekly and the numbers of cocoons produced per week were assessed. Fecundity, growth, maturation and the biomass production were all significantly greater at 25°C than 15°, 20° or 30°. The growth of individual earthworms increased at the lower population density, but the greatest overall earthworm biomass production occurred at the highest population density. The highest ratio of conversion from organic waste to earthworm biomass (dry weight) was 10:1. Cocoon production, the time for cocoons to hatch, the percentage hatch and the number of earthworms hatching per cocoon, at each temperature were recorded. The rate of growth of hatchlings at these temperatures was measured. The greatest number of cocoons per week and the number of hatchlings per cocoon were obtained at 25°C. Cocoons of *E.*
*Eugenia eugeniae* hatched in only 12 days at 25°C, the earthworms at these temperatures reached sexual maturity in as little as 35 days after hatching and gained weight at maximum rate of 280 mg per week. Cocoon production differed markedly in relation to temperature and the number of cocoons produced by *E. eugeniae* was significantly higher at 25°C than at 20°C with relatively few cocoons produced at 15°C and 30°C. The largest numbers of cocoons were produced at 25°C and at this temperature, the hatching percentage and the number of hatchlings produced per mature earthworm were also significantly higher. These same indices of reproduction success were very low in cultures kept at 30°C. *Eudrilus eugeniae* increased in total biomass much more rapidly than *E. fetida*, a species which grows relatively well in most organic wastes. (Domínguez et al. 2001). Moreover, *E. eugeniae* reached sexual maturity in as little as five weeks compared with *E. fetida* which took 6-8 weeks to produce its first cocoon (Edwards, 1988). The maximum weight gain of *E. eugeniae* was 280 mg per week, compared to the highest weight gains reported for *E. fetida* of 60-80 mg per week (Watanabe and Tsukamoto, 1976). In rainy season worms produced cocoons from 21st to 60th day and in summer season cocoon productions were initiated in the 60th day to 80th day. During winter season worms took 100 days as their active period of activity and produced cocoons from 60th to 100th day period. Favourable and steady growth and reproduction was attained during winter season and the other two seasonal impact on the worms were seen as early and extended reproductive stages (in rainy season) and extended growth stages of large immatures (in summer season) with only last 20 days which were reproductively active days.

Numerous reproductive parameters have been studied in earthworms exposed to various xenobiotics: cocoon and hatchling production, viability of the worms produced, and sexual maturation. Cocoon production was found to be the most sensitive parameter for paraquat, fentin, benomyl, phenmedipham, carbaryl, copper oxychloride, dieldrin, while cocoon hatchability was most sensitive for pentachlorophenol, parathion and carbendazim, copper oxychloride. Bustos-Obregón and Goicochea (2002) explored the effect of exposure to commercial parathion on the reproductive parameters such as sperm and cocoon production and genotoxicity on male germ cells of *Eisenia fetida* and reported that alterations in reproductive parameters were conspicuous in regard to the number of sperm, cocoons, and worms
Numbers of juveniles per cocoon can be regarded as sensitive parameters to evaluate the toxicity of acetochlor on earthworms as reported by Xiao et al. (2006). Choo and Baker (1998) also found that cocoon production in *Aporrectodea trapezoides* was inhibited by endosulfan and fenamiphos at normal application rates and methiocarb at 10× normal rate.

Espinoza-Navarro and Bustos-Obregón (2005) treated *Eisenia fetida* with organophosphate insecticide malathion and found that malathion decreased the spermatic viability in spermatheca, altering the cell proliferation and modifying the DNA structure of spermatogonia. Sperm count also seems to be a very sensitive marker, malathion could affect the sperm count, but in addition, its metabolites could affect sperm quality. Several scientists have reported that pesticides influence the reproduction (cocoon production, a reduced mean and maximum number of hatchlings per cocoon, and a longer incubation time) of worms in a dose-dependent manner, with greater impact at higher concentration of chemical. Gupta and Saxena (2003) studied the effects of carbaryl, an N-methyl carbamate insecticide, on the reproductive profiles of the earthworm, *Metaphire posthuma* and found sperm head abnormalities even at the lowest test concentration of 0.125 mg/kg. Wavy head abnormalities were observed at 0.125 mg/kg carbaryl, whereas at 0.25 mg/kg and 0.5 mg/kg, the sperm heads became amorphous and the head nucleus was turned into granules deposited within the wavy head.

The number of cocoons varied significantly with the season. Similarly, the rate of cocoon production also varied significantly. The average rate of cocoon production was 7.23, 0.99 and 0.53/worm/week in the monsoon season, winter and the summer, respectively (Biradar et al. 1999). Maximum mean weight of the adult earthworms was achieved in winter, at the lowest temperatures, but these large-sized earthworms showed the lowest mating activity during this period (Kaplan et. al. 1980). The waste decomposition and the earthworm production was associated strongly with the quality of the substrate especially with the chemical as well as biological composition of the substrate (Suthar, 2006). The increase in nitrogen content during vermicomposting is due to the nitrogen release by the earthworm’s metabolic process and the dead tissues (Araujo et al. 2004) The organic carbon is lost as carbon dioxide and nitrogen increases as a result of carbon loss (Crawford, 1983). Microorganism that use the
carbon as a source of energy and Nitrogen for building up their cell structure brings about decomposition of the organic matter. But the reduction is greater in vermicomposting as compared to the ordinary composting. This may be due to the fact that the earthworms have a higher assimilating capacity. The increase in the nitrogen value is as result of carbon loss and probably because of mineralization of organic matter (Kaushik and Garg, 2003).

Xiao et al. (2006) showed that acetochlor had no long-term effect on the reproduction of *Eisenia fetida* at field dose (5–10 mg/kg). At higher concentrations, acetochlor (20–80 mg/kg) revealed sublethal toxicity to *Eisenia fetida*. Zhou et al. (2008) assessed and found chlorpyrifos had adverse effect on fecundity in earthworm exposed to 5 mg/kg chlorpyrifos after eight weeks. According to Zhou et al. (2008), reproduction of earthworms appeared to be more severely affected by cypermethrin at juvenile stage than at adult stage. Application of 20 mg/kg, cypermethrin caused significant toxic effects in reproduction of worms. Coiling seen in the parathion treated worms, interferes with the reproduction too since worms find their partner less easily and copulation is abnormal in terms of mating posture. Ejection of sperm seems also to be hindered and therefore a large number of spermatozoa are found in intoxicated worms in spite of a clear effect on sperm production under parathion treatment as discussed by Bustos-Obregón and Goicochea (2002). According to Espinoza-Navarro and Bustos-Obregon (2004) malathion also has a direct cytotoxic effect causing coiling of the tail, with increase of metachromasia of the chromatin of the spermatozoa and altering the sperm count.

Lapinski and Rosciszewska (2008) in their study exposed *Eisenia fetida* Sav. earthworms to a series of increasing concentrations of cadmium and mercury. The strongest impact of substrate contamination was exerted upon the number of young individuals and cocoons. The cadmium contamination did not affect adversely the mass of earthworms, whereas in the mercury contaminated group the decline in body mass was evident. After 8 months of experiments, the content of heavy metal in the bodies of earthworms was determined. An evident relationship between the cadmium and mercury contents in the substrate and their accumulation in earthworms’ tissues was found. Žaltauskaitė and Sodienė (2010) studied the effects of total cadmium and lead concentrations in soil on the growth, reproduction and survival of earthworm
Eisenia fetida. The weight of earthworms declined in all treatments during the experiment. Earthworm weight negatively correlated with lead concentrations in soil, however the weight of the earthworms exposed to cadmium tended to show a dose-related decrease. Cocoon production was more sensitive than mortality and growth. A regression analysis showed that cocoon production rate significantly decreased with increasing lead concentration in soil and Cd inhibited the cocoon production rate by 18–65%.

Effect of Heavy Metal on the Bio Molecule Constituents of Different Species.

Dominguez et al. (2001) showed that Eisenia fetida after exposures to cadmium (Cd), zinc (Zn) or cadmium + zinc spiked media. MT (microtubules) was studied both at the protein level by Dot Immunobinding Assay, (DIA) and at the expression level by Northern blotting. Cd was highly accumulated by worms whereas Zn body concentration was regulated. In addition, Zn would limit Cd accumulation in worms exposed to low Cd concentrations (1 and 8 mg Cd/kg of dry soil). Exposure to a mixture of Cd and Zn at high concentrations increased cytosolic MT levels. This increase would allow worms to regulate body Zn concentrations and also to limit Cd toxicity. Cd exposures increased gene expression of Cd-binding MT isoform (MT 2A) whereas Zn did not. However, when both metals were at high concentrations in the exposure medium, this expression was further increased. Several hypotheses are proposed to explain the results and the best approach to estimate metal exposure of this earthworm species. Further experiments have now to be performed to evaluate the usefulness of these MT responses for field contaminated soils toxicity assessment using this earthworm species. Cd, Cu and Zn concentrations in soil, pore water and CaCl2 extracts of soil, in leaves of the plant species Urtica dioica and in earthworms were determined at 15 field sites constituting a gradient in metal pollution. Variations in the Cu and Cd concentrations in L. rubellus and Cu concentrations in A. caliginosa were best explained by total soil concentrations, while variation in Cd concentration in A. caliginosa was best explained by pore water concentrations. Zn concentrations in L. rubellus and A. caliginosa were not significantly correlated to any determined variable. Despite low availability, earthworms in floodplain soils contain elevated
concentrations of Cu and Cd, suggesting that uptake takes place not only from the soluble metal concentrations.

Xiao et. al. (2006) showed the effects of earthworm (*Eisenia fetida*) activity on soil pH, dissolved organic carbon (DOC), microbial populations, fraction distribution and bioavailability of heavy metals (Zn, Cu, Cr, Cd, Co, Ni, and Pb) in five Chinese soils using pot experiments. A three-step extraction procedure recommended by the European Community Bureau of Reference (BCR; now Standards, Measurements and Testing Programme of the European Community) was used to fractionate the metals in soils into water soluble, exchangeable and carbonate bound (B1), Fe-oxides and Mn-oxides bound (B2) and organic matter and sulfide bound (B3). After the soils were treated with earthworms, the soil pH, watersoluble metal fraction and DOC increased. A significant correlation was obtained between the increased DOC and the increased metals in the water-soluble fraction. The heavy metals in fraction B1 increased after earthworm treatments, while those in fraction B3 decreased. No significant differences were observed for heavy metals in fraction B2. The microbial populations in soil were enumerated with the dilution plate method using several media in the presence of earthworms. The microbial populations increased due to earthworm activity. The biomass of wheat shoots and roots, and the heavy metal concentrations in wheat roots and shoots, were also increased due to the earthworm activity.