EFFECTS OF PAPER MILL SLUDGE ON ACETYLCHOLINE ESTERASE ACTIVITY DURING THE PROCESS OF VERMICOMPOSTING

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***Abstract*:** Paper making processes in industries discharge heaps of sludge to their surrounding areas leading to soil contamination. The chemicals present in the sludge may affect the metabolic activity of the earthworms, which plays an essential role in degrading the organic waste into valuable nutrient for maintaining soil productivity. Acetylcholinesterase (AChE) is a key enzyme in the nervous system, terminating nerve impulses by catalyzing the hydrolysis of neurotransmitter acetylcholine. AChE inhibition in earthworms is presently regarded as giving early warning of adverse effects of pollutants. Therefore, to assess the effects of paper mill sludge (PMS), on the metabolic activity of earthworms, AChE activity of the earthworms was studied during the vermicomposting of PMS. For the experiment five sets of cement rings of diameter 2ft. and 1ft.height were taken with four replicates each. In each cement ring 80kg of soil and sludge mixture was taken in various concentrations. Each bin of the first set i.e. the control set contained only powdered soil, and the rest other bins of second, third, fourth & fifth sets contained powdered soil and sludge mixture in the ratio of 25% sludge & 75% soil, 50% sludge and 50% soil, 75%sludge & 25% soil and 100% sludge respectively. The soil and sludge mixture in the rings were maintained with 40±5gm% of moisture and 25±5°Ctemperature. AChE activity was measured with respect to paper mill sludge at the interval of 15 days for 180 days. Higher AChE activity i.e.8.18µmole AChE hydrolysed/ mg tissue/hr was observed in 50% sludge composting than other concentrations.

Key words: **Acetylcholinesterase (AChE) enzyme, paper mill sludge, vermicomposting**

I. INTRODUCTION:

Industrial expansion has led to the production of enormous quantity of organic waste. Paper mill industries are such industries which discharge heaps of sludge to their surrounding areas during paper making processes leading to soil contamination. This sludge contains chemicals (Suriyanarayanan et al 2010) which may affect the activity of some beneficial organisms. Earthworms are one of the important members, playing major role in the development and maintenance of soil structure (Edwards and Bohlen 1996) by breaking organic matter and releasing plant nutrient (Bartlett et al 2010) and is also a source of food for other organisms (Paoletti 1999; Jongmans et al 2003). Numbers of literature are available to support the splendid work of the earthworm to degrade the biodegradable organic waste released from the industries into high quality manure, which are essential for maintaining soil productivity (Bhiday 1994; Suthar 2005; Aishwariya and Amsamani 2012; Kaouachi et al 2013; Bhat et al 2015; Anusha and Paul 2015; Sosnecka 2016;). As these earthworms have strong interaction with soil, they are profoundly affected by pollutants coming from intensive use of biocides in agriculture, industrial activities, and atmospheric deposition (Lionetto et al 2011). No, doubt earthworms have the ability to tolerate many kinds of chemical contaminants (Corp and Morgan 1991; Spurgeon and Hopkin 1999) but the tolerating capacity decreases with the increasing percentage of the

contaminants present in the soil. Paper mill sludge is rich in organic matter and plant nutrients (Dayegamiye et al 2002) but directly disposing these wastes may have an adverse impact on soil quality and metabolic activity of the earthworms due to the presence of chemicals in it. Generally, earthworms are sensitive to soil disturbances; therefore, application of overdoses of fertilisers and industrial wastes as soil amendments is likely to adversely impact these animals (Samal et al 2017). Many researchers have suggested that industrial organic waste mixed with organic substance can be potentially utilized by the process of vermicomposting (Melgar et al 2009; Padmabati 2013; Sepperumal and Selvanayagam 2015) Paper mill sludge is not able to support earthworms growth by itself, when mixed with organic matter could be suitable for vermicomposting (Elvira et al 1996; Elvira et al 1997; Elvira et al 1998). This ultimately decreases the percentage of chemicals which could be healthier on the part of earthworms for the process of composting, giving double benefit i.e. to produce nutrient rich vermicompost and to reduce the pollution due to paper mill sludge. Acetylcholine esterase (AChE) (E.C.3.1.1.7.) is one of the important enzymes and plays an essential role in acetyl choline-mediated neurotransmission (Lionetto et al 2011) in the vertebrate and invertebrate nervous system (Tommaso et al 2017). It is present in the cholinergic synapses in the central nervous system as well as in neuromuscular synapses where it rapidly hydrolyzes acetylcholine (Jha and Rizvi 2009). Inhibition of the AChE enzyme results in accumulation of acetyl choline causing a continuous and excessive stimulation of the nerve/muscle fibres (Dutta & Arends, 2003) which leads to tetany, paralysis and eventual death of the organisms (Lionetto et al 2011). Therefore, monitoring of AChE inhibition is widely used as an important parameter (Dembele et al 1999) and is frequently used as a biomarker of effects of neurotoxic contaminants (Galgani et al 1992; McHenery et al 1997 ; Kirby et al 2000) like insecticide and pesticide, heavy metals, detergents, hydrocarbons, herbicides and industrial pollutants. Therefore, the objective of the current study was to measure the effect of paper mill sludge on the metabolic activity of the earthworm *Lampito mauritii*. This has been done by assessing the AChE activity of earthworms during the period of composting of paper mill sludge mixed with soil in different percentage.

II. MATERIALS AND METHODS:

In the present study dried bulks of paper mill sludge was collected from the dumping sites of Emami Paper Mill located at Balgopalpur, Balasore, Odisha, India. It is a paper producing industry which uses waste papers as raw materials. Cement rings of diameter 2ft. and 1ft. height each were taken in 5 sets with 4 replicates in each set. Each bin of the first set contained 80kg of dried powdered soil without any amendment, which was called as control set, the second set contained 25% sludge and 75% soil i.e. 20kg of powdered sludge mixed with 60kg of powdered soil, the third set contained 50% sludge and 50% soil i.e. 40kg of sludge mixed with 40kg of soil, the fourth set contained 75% sludge and 25%soil i.e.60kg of sludge mixed with 20kg of soil, the fifth set contained only sludge which was called as 100% sludge. Forty numbers of matured earthworms of species *Lampito mauritii* were released into each bin of all the sets. The composting bins were maintained with 40±5gm % of moisture. Soil samples from each bin were collected at the interval of 15 days for a period of 180 days for measuring Acetylcholinesterase (AChE) activity of the earthworm following spectrophotometric method of Glick (1957). It was expressed in terms of acetylcholine hydrolysed per milligram body tissue per hour. The mean values were depicted in Fig- 1.

III. RESULTS AND DISCUSSION:

Figure 1- presents AChE (Acetylcholinesterase) activity in earth worm collected from the soil amended with paper mill sludge in different concentration during the process of vermicomposting. In control maximum AChE activity was observed to be 6.02 on 180th day. In 25% sludge composting AChE activity increased gradually from 4.32 to 7.98. Similarly, in 50% sludge, 75% sludge, & 100% sludge composting, AChE activity increased gradually throughout the composting period. Maximum AChE activity in earth worm was found to be in 50% sludge composting *i*.e 8.18 on 180th day.

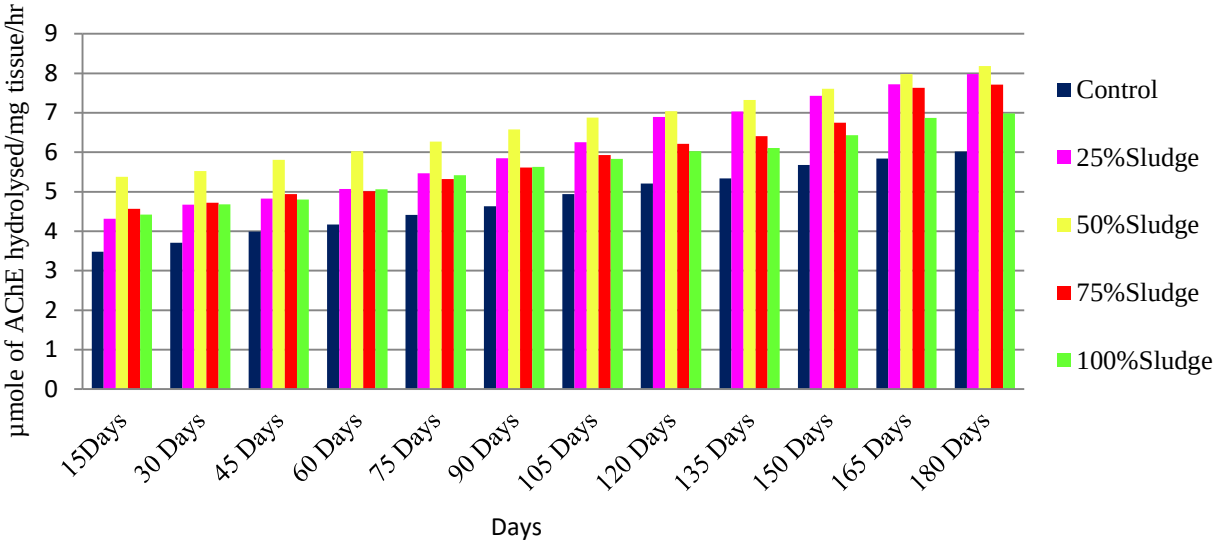

Fig.1. AChE activity of earthworms during the process of vermicomposting in different concentration of sludge

Acetylcholinesterase represents the main cholinesterase in earthworms (Thompson and Richardson 2004; Rault et al 2007). Its activity has been identified and biochemically characterised only in a few earthworm species (Caselli et al 2006). It has also been found that the concentration of AChE activity was highest in the pre-clitellar part of the animal and has a main role in functioning of the dorsal brain localized near the prostomium (Calisi et al 2011a). Presently, AChE inhibition in earthworms is regarded as a biomarker to assess the impact of pollutant on wildlife in terrestrial ecosystems (Booth & O'Halloran 2001). Various workers (O'Brien RD 1967; Guilhermino *et al* 1996; Rao et al 2003; Denoyelle et al 2007; Bednarska et al 2017) have demonstrated that AChE activity is inhibited in earthworms exposed to various groups of insecticides. Many reports are also available where inhibition of AChE in earthworms was observed when exposed to pesticides, both in laboratory tests and under field conditions (Rao et al 2003; Schreck et al 2008; Calisi et al 2009; Scott-Fordsmand & Weeks 2000; Rao and Kavitha 2004; Gambi et al 2007). Similarly, depressed activity of AChE in earthworms has been demonstrated in several studies with respect to heavy metals (Tommaso et al 2017; Bednarska et al 2017). Pattanayak *et al* (2013) also reported inhibition of AChE activity of *Octochaetona surensis* at all the applied concentrations of Cr and Ni in soil, compared to control soil, under laboratory conditions. Likewise, decline in enzyme activity in *L. mauritii* with exposure to medium and high treatment concentrations of Phosphogypsum and Paper Mill Sludge was observed (Samal et al 2017). On the contrary low levels of metal treatments on the earthworm *Lampito mauritii* do not apparently effect the activity of acetylcholinesterase (Maity et al 2010). Work done by (Calisi et al 2011b) in *Lumbricus terrestris a*nd (Calisi et al 2009) in *Eisenia fetida indicated that AChE* was unaffected by copper sulphate exposure. But, an enhanced enzyme activity was observed at all concentrations in Lampito mauritii exposure to urea (Samal et al 2017) thus agreeing with our findings. The present study also indicated that the AChE activity to be more in all the concentration of sludge compost than control soil. With increase in sludge concentration i.e.(75% & 100%sludge) the AChE activity was decreased. It showed that the enzyme activity was suppressed at high concentrations of sludge indicating that higher percentage of sludge acted as AChE inhibitor leading to an accumulation of acetylcholine at central cholinergic synapses and neuromuscular junctions in *Lampito mauritii.* Maximum AChE activity was observed in 50% sludge condition suggesting this concentration of sludge to be favourable for the metabolic activity of the earthworm.

CONCLUSION:

Paper mills industries disposing sludge in the open areas has increasingly become a matter of concern. The accumulation of sludge in soil is believed to affect the soil fertility and the population of biota. Evidences from the

present study confirms that, when earthworms are exposed to different concentration of paper mill sludge, maximum AChE activity was found to be in 50% sludge concentration. Higher concentration of sludge inhibits AChE activity in earthworms. Therefore a mixture of 50% sludge and 50% soil proved to be effective for the process of vermicomposting. The results confirmed that, the vermicomposting as an alternative technology for the management of paper mill sludge if mixed with soil in appropriate proportion.

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