ORIGINAL RESEARCH PAPER

Zoology

KEY WORDS: Phosphogypsum, Fertilizer, Eisenia, Population

EFFECT OF PHOSPHOGYPSUM ON GROWTH AND REGENERATION OF EARTHWORM WITH SPECIAL REFERENCE TO *"Eisenia fetida"*

Asit Kumar Behera School of Life Sciences, Sambalpur University, JyotiVihar, Burla.

Dr. AlivaPatnaik School of Life Sciences, Sambalpur University, JyotiVihar, Burla.

Chetna Mishra School of Life Sciences, Sambalpur University, JyotiVihar, Burla.

Monalisa Chhatria School of Life Sciences, Sambalpur University, JyotiVihar, Burla.

ABSTRACT

The majestic and diverse nature of terrestrial ecosystem allows to inhabit a wide and diverse range of flora and fauna. But the ironyis speedy increase ofindustries and industrialization hinders/for-fend to achieve comely environment forflora and fauna to flourish and these empathy regarding environmental pollution just made it count for the destructive consequences in near future. Phosphogypsum (PG) is one of hazardous by-product produced form fertilizer plants and its chemical constituents may hamper the inhabiting soil dwelling organisms. In this investigation we focused on the impact of PG on growth and regeneration of Earthworm Eisenia fetida. Study reveals about the fatal nature of PG where growth and regenerationadversely affected by the increase of dose concentration (0 g %, 4 g %, 8 g % and 10 g %) and duration (10 days, 20 days and 30 days).

INTRODUCTION :

Versatility and flexibility of terrestrial ecosystem and its excellent accommodating features for soil dwelling organisms (Micro, Meso and Macro) becomes a great matter of concern for ecologist nowadays. The extraordinary and diverse features of terrestrial ecosystem in particular soil nowin threatened state.Population explosion and its rapid increase of food demand exert immense and immediate impact over soil ecosystem and its inhabiting organisms. Industry and industrialization and its abysmal consequences had been noticed very long before. Various waste and by-products of industrial sectors affects soil ecosystem due to lack of proper disposal methods. Phosphogypsum (PG) one of the by-product of fertilizer industries has the corrosive impact upon soil biota. Generally PG is composed of heavy metals (Cadmium, Cromium, Lead, Zinc, Mercury, etc.) and radioactive nuclei (Uranium and Thorium) (Hazardous waste management series: HAZWAMS/2012-2013). Among various soil dwelling animals earthworms are much more involved with soil formation, alteration and fertility and it can considered as key stone species of soil ecosystem. It has the fantastic capability to adapt with fluctuating environment and retain the integrity of soil. More importantly, earthworms can accumulate the heavy metals to a greater extent and can act a pollution indicator. But the dilemma is how far and how much they can accumulate.

Eisenia fetida is one of the rapidly growing (Neuhauser *et al.*, 1980) and prodigiously reproducing earthworm (Hartenstein *et. al.*, 1979). It has the ability to regenerate lost segments. *Eisenia fetida* with head regeneration, in an anterior direction, possible at each intersegmentallevel back to and including 23/24, while tails were regenerated at any levels behind 20/21, i.e., twoworms may grow from one (Gate, 1949). It is potentially deployable for management of wastes rich in microbial biomass (Hartenstein, 1981). It is an epigeic earthworm and mostly used for vermi-composting.The hardy nature of this worm canhelp in tolerating wide fluctuation of temperature and humidity and this particular feature enables easy culturing of thisspecies. Present investigation aimed to determine the actual impact of PG on growth and regeneration of *Eisenia fetida* in different concentration doses with respect to duration of exposure.

Materials and methods -:

Phosphogypsum (PG) : One of the by-product ofDi-Ammonium Phosphate (DAP) fertilizer industries. Gypsum used in the production of DAP fertilizer which ultimately gives Phosph ogypsum as a by-product.

Phosphate ore (Gypsum) + sulfuric acid = DAP + Phosphogypsum
Gypsum was collected from Paradeep Phosphate Limited (PPL), Paradeep.

Earthworm Used : *Eisenia fetida* used as experimental organisms. For regeneration adult earthworms with prominent clitellum were used.

Soil : Soil was sieved using 2 mm sieve and packets were made containing 0g%, 4g%, 8g% and 10g% gypsum with 25 g% water.

Method: 500 gm 2 mm sieved, air dried soil was taken in polythene packets with moisture maintained at 25 gm % by addition of distilled water in all packets. The control consisted of 500g soil only and PG was added to soil in experimental sets i.e. 4%, 8% and 10%. Five replicates of each concentration were taken. After moisture addition the packets were left for five days for stabilization. Culture was maintained with 25 g% moisture and 25 oC temperature. For regeneration, the guts of the matured earthworm were cleaned and about 50% of post clitellar region was cut with sharp blade. Five earthworms were inoculated into each packet after amputation. Thus twenty five earthworms each were cultured in control, 4%, 8% and 10% of gypsum. For growth up to 1.70 gm were inoculated to each packet after five days of moisture addition during which microbial activation of the soil was done with temperature maintained at 25 0C .The number of segments regenerated and weight of earthworm in each packet were counted/determined on 10th 20th and 30th days. The earthworms after amputation could not survive in 8 % and 10 % PG. So the regeneration experiment was conducted with 4 % PG only. Significant difference due to gypsum was analyzed by using t- test.The change in the weight over initial weight was observed after 10, 20 and 30 days and percentage change in weight of earthworms over zero day culture was estimated. Statistical analysis, ANOVA was performed to infer the results (Gupta, 1980).

RESULT-:

Growth : After 10 days there was a positive increase of about 1.53% in weight in control set ups in comparison to PG exposure. On exposure to 4 %, 8 %, and 10 % PG, there was decrease of about 23.07 %, 23.07 % and 75 % respectively over zero day was observed. At 20 days of exposure an increase in weight of about 5.03 % was observed over zero day in control but a continuous decrease in weight by 33.84 %, 68.46 % and 93.84 % was observed when exposed to 4 %, 8 % and 10 % of PG. After one month of exposure to 10 % PG there was about 96.40 % decrease in weight over initial weight. Their weight was decreased by 30.93 and 74.82 % over initial weight on exposure to 4 % and 8 %, respectively on 30th day and there was an increase of 21.53% in weight in control over zero day. Analysis of variance showed significant impact of PG treatment on earthworm weight at 0.05 level of significance.

Table 1: Weight of *Eisenia fetida* earthworm under impact of Phospho gypsum (PG) in laboratory culture

Culture in Days	Parameter Analysed	Conc. Of Phosphogypsum in %			
		0	4	8	10
0	Weight % change over '0' day	1.01 0	1.03 0	1.05 0	1.02 0
10	Weight % change over '0' day	1.04 +1.53	0.8 -23.07	0.8 -23.07	0.26 -75.0
20	Weight % change over '0' day	1.3 +5.3	0.86 -33.84	0.41 -68.46	0.08 -93.84
30	Weight % change over '0' day	1.39 +21.53	0.96 -30.93	0.35 -74.82	0.05 -96.40

Figure 1: Change in weight of Eisenia fetida earthworm under the impact of Phosphogypsum (PG) in laboratory culture

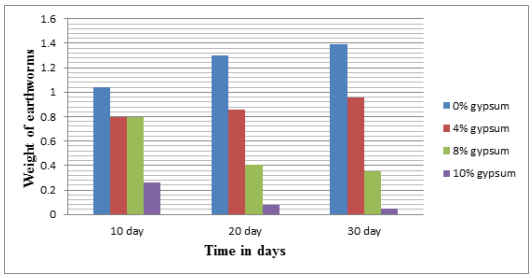
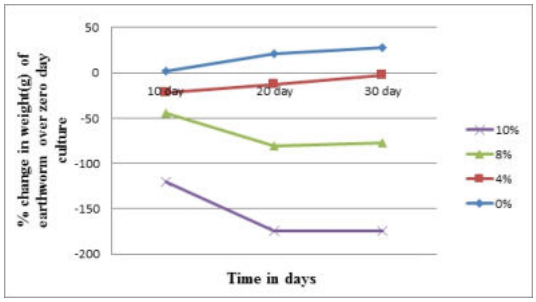


Figure 2: Percentage change in weight of earthworm Eisenia fetida under the impact of Phosphogypsum (PG) in laboratory cultures



Regeneration : The amputated earthworms could not survive beyond two days in 8% and 10% gypsum. So the experiment was continued with control and 4% gypsum. After 10 days of exposure to 0 g % and 4 g % of gypsum, the percentage increase in regenerated segments was found to be 6.28 % and 2.68 % respectively. After 20 days of exposure to 0 g % and 4 g % gypsum, the percentage of regenerated segments was found to be 10.64 and 5.72 % respectively. At the end of 30 days of exposure to 0 g % and 4 g % of gypsum the percentage increase in regenerated segments was found to be 13.4 and 8.4 %. T - test revealed level of significance at 0.05 level was significant.

Table – 2: Regeneration of *Eisenia fetida* earthworm under the impact of Phosphogypsum (PG) in laboratory culture

Culture in days	Parameters analysed	Conc. Of Gypsum in g %	
		control	4
10	Total no. of segments regenerated	6.28	2.68
	%age change over control	0.0	57.32
20	Total no. of segments regenerated	10.64	5.72
	%age change over control	0.0	46.24
30	Total no. of segments regenerated	13.4	8.4
	%age change over control	0.0	37.31

Fig – 3, Percentage change in regeneration of *Eisenia fetida* earthworm under the impact of Phosphogypsum (PG) over 0 g % in laboratory culture

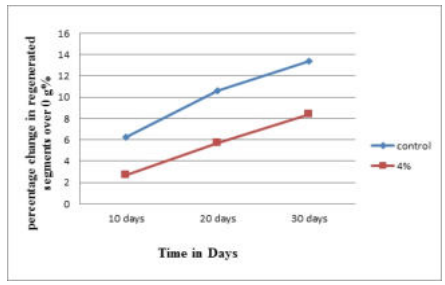


Figure 4: Regenerated segments of earthworm in 4% Phosphogypsum(PG)

DISCUSSION - :

After the exposure of dose concentration the growth of earthworm decreases with increase of PG. Farruk and Ali (2011) clearly showed that dichlorovos caused a decrease in weight of all groups of earthworms, when they were exposed to different concentration of dichlorovos fumigant insecticide. Yasmin and D'souza (2007) investigated the impact of carbendazium, glyphosphate and diamethoate on Eisenia fetida and found a significant reduction in the earthworm growth in a dose-dependent manner. Choo and Baker (1998) found endosulfan did significantly reduce the weight of juvenile Aporrectodea trapezoids within 5 weeks when applied to soil at normal application rate in the field only. Podalok et al., (2011) reported that body weight and number reduced due to impact of heavy metals. Fordsmand et al., (1998) studied the impact of heavy metal in particular nickel on survival, growth and reproduction of Eisenia venta earthworm and concluded that nickel has a very profound effect. In an another study Zigmontien and Liberyt (2014) have shown that a large amount of heavy metals (Cr, Ni, Cd etc.) accumulated by earthworms from sewage sludge. Kaur and Hundal (2016) reported that presence of heavy metal in soils can adversely affects the growth, reproduction and development of E. fetida. Spurgeon et al., (1992) concluded about the clear and profound impact of heavy metals i.e Cd, Cr, Cu and Zn on growth, reproduction and survival of E. fetida. Weight of earthworms declined consistently by the increase of exposure period.

Regaining the lost parts of the body is regeneration and it is proportional to growth. As it involves the reactivation of cells at short notice, repair of damage is a traumatic stress, the regeneration is a more complicated process than normal growth (Stephensons, 1930). Inhibition of regeneration on application of mercury in Eisenia fetida has been reported before (Beyer et al., 1985). In the present studies regeneration rate has been significantly reduced by application of PG at sub-lethal level. Increase in the loss of energy in maintenance cost might be important factor regarding regeneration. Patnaik (2013), has reported reduction in rate of regeneration on application of nickel in sub-lethal doses.

CONCLUSION -:

In present study significant reduction in weight of the earthworm was found on exposure to sub-lethal dose to PG. Growth rate is decreasing as most of the energy directed towards the respiration. Regeneration of cut part in earthworms was observed very little in comparison to control and it was noticed, PG has a profound and abysmal impact upon regeneration capacity of E.fetida as the chemical composition of PG has an immense impact upon the physiology and sustainability of E.fetida. Heavy metals can

accumulate by the earthworms to a limited extent where they can also useful as an indicator of pollution. But if the concentration of heavy metals and pollution level exceeding the survival range and more than the sub-lethal dose then neither earthworm can accumulate the heavy metals nor used as a pollution indicator. Soil health and its fertility depend upon macro-faunas particularly earthworms and the integrity revolved around earthworms. So it can summed up with soul of soil is earthworm. We need to generalize these vital facts of soil and should be more aware about the importance and contribution of Earthworms towards soil and terrestrial ecosystem.

REFERENCES

1. Beyer W.N., Cromartie E. and Moment G.B. (1885). Accumulation of methylmercury in the earthworm, Eisenia fetida, and its effect on regeneration. Bulletin of environmental contamination and toxicology, 35(1): 157-162.
2. Central pollution control board (2012). Hazardous waste management series: HAZWAMS/2012-2013.
3. Choo L.P.D. and Baker G.H.(1998). Influence of four commonly used pesticides on the survival, growth and reproduction of the earthworm Aporrectodea trapezoids (Lumbricidea). Australian journal of agricultural Research, 49(8):1297-1303.
4. Farrukh S. and Ali A.S. (2011). Effects of dichlorovos organophosphate on growth, reproduction, and avoidance behaviour of earthworm Eisenia fetida. Iranian journal of toxicology 5(14): 495-501.
5. Fordsmand J. J. S., Weeks J. M. and Hopkin S.P. (1998). Toxicity of nickel to the earthworm and the applicability of the neutral red retention assay. Ecotoxicology, 7(5): 291–295.
6. Gate G.E. (1949). Article "Miscellanea Megadrilogica". DOI: 10.1086/281596.
7. Gupta S.P. (1980). Statistical Methods (14theds). Sultan Chand and Sons. New Delhi, pp 1000.
8. Hartenstein R. and Hartenstein F. (1981). Physicochemical changes effect in activated sludge by the earthworm Eisenia fetida. J. environ. qual., 10: 377–382.
9. Hartenstein R., Neuhauser E.F. and Kaplan D.L. (1979). Reproductive potential of the earthworm Eisenia fetida. Oecologia (Berl.) 43: 329–340.
10. Hartenstein R., Neuhauser E.F. and Narahara A. (1981). Effects of heavy metal and other elemental additives to activated sludge on growth of Eisenia fetida. J. environ. qual., 10 : 372–376.
11. Kaur K. and Sangha G.K.(2014). Effect of metal contaminated soil on Eisenia fetida (savigny) at Ludhiana (Punjab), India. Journal of applied and natural sciences, 6(2): 519-523.
12. Neuhauser E.F., Hartehstein R. and Kaplan D.L. (1980). Growth of earthworm Eisenia fetida in relation to population density and food ratioing. OIKOS, 35: 93-98.
13. Patnaik A. and Behera M.K. (2013). Sublethal impact of nickel on survival and soil metabolism of an anecic earthworm. International journal of scientific research, 2(12): 106-109.
14. Podolak A., Piotrowska E., Klimek M., Klimek B. A., Kruk J. and Plytycz B. (2011). Effects of nickel, zinc, and lead contaminated soil on burrowing rate and coelomocytes of the earthworm, Allolobophora chlorotica. Folia Biol (Krakow), 59: (34) – 917.
15. Spurgeon D. J., Hopkin S. P. and Jones D. T. (1992). Effects of cadmium, copper, lead and zinc on growth, reproduction and survival of the earthworm Eisenia fetida (savigny): assessing the environmental impact of point-source metal contamination in terrestrial ecosystems. Environmental pollution 84: 123-130.
16. Spurgeon D.J. and Hopkin S.P. (1996). Effect of metal contaminated soil on the growth, sexual development and early cocoon production of earthworm Eisenia fetida, with particular reference to zinc. Ecotoxiology and enviornmental safety, 35: 86-95.
17. Spurgeon D.J. and Hopkin S.P. (1998). Comparison of metal accumulation and excretion kinetics in earthworms (Eisenia fetida) exposed to contaminated field and laboratory soils. Applied soil ecology, 11: 227-243.
18. Spurgeon, D.J. and Hopkin, S.P. (1996). The effects of metal contamination on earthworm population around smelting areas quantifying species effects. Applied soil ecology 4: 147-160.
19. Stephenson J. (1930). The Oligochaeta. Oxford Univ. Press.
20. Yasmin S and D'Souza D. (2010). Effect of pesticides on growth and reproduction of earthworm: A Review. Applied and environmental soil science, volume 2010, Article ID 678360, 9 pages.
21. Zigmontiene A. and Liberyte I. (2014). Heavy metals (Cr, Cd and Ni) concentrations in sewage sludge and bioaccumulation by californian earthworms in the process of vermicomposting. 9th international conference on "environmental engineering".