NEGATIVE EFFECT OF PHOSPHOGYPSUM OVER PHYSIOLOGICAL ACTIVITY OF EARTHWORM *EISENIA FETIDA*

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(Received 22nd Mar 2018; accepted 27th Jun 2018)

Abstract. The study aimed to assess the genuine impact of phosphogypsum on the growth, feeding, respiration and regeneration of earthworm *Eisenia fetida*. In laboratory condition the earthworms were cultured under 0% (control), 4%, 8% and 10% concentration of phosphogypsum for 30 days. After completion of every 10 day changes in the above parameters were observed to track the impact of phosphogypsum. With increasing exposure duration and concentration of phosphogypsum lower growth rate, declined feeding habit, maximum respiration rate and deprived regeneration power were noticed. The highest and lowest growths were 1.39 gm at 0% and 0.05 gm at 10%, respectively. Maximum feeding rate was 32.65 with a minimum rate 16.20 g g^{-1} live tissue. Respiration rate was highest at 10% i.e. 0.0578 g^{-1} live worm tissue hr^{-1} kg^{-1} soil, as most of the energy used to respire to sustain in such diverse condition and 0.575 g^{-1} live worm tissue hr^{-1} kg^{-1} soil recorded as lowest in 0%. The rate of regeneration was deeply hampered and there was no viable worms left at 8% and 10% concentration to assess. Regeneration was only observed at 0% and 4%.

Keywords: *growth, feeding, respiration, regeneration, earthworm, concentration*

Introduction

Anthropogenic activity and environmental changes are the major sources which directly affect the integrity of terrestrial ecosystems. Industrial activities, waste disposal and agricultural practices are sources of soil pollution. Soils are complex associations containing living organisms, mineral particles and organic matter. The clay fractions of the minerals and the humus of organic matter are colloids of a very small size with a large surface area to volume ratios, and consequently with a high binding capacity to inorganic and organic molecules. The binding of pollutants to soil colloids reduces their mobility and their bioavailability and modulates their biological effects. Deposition of different contaminants on soil through anthropogenic sources are very much prone to alter the basic composition and this might be hazardous to inhabiting organisms. The macrofaunal groups of soil are the integral part of the soil ecosystem and have a significant role in soil fertility and functioning. Earthworms are the one of the macrofaunal inhabitant of the soil and hold the key role in soil formation, alternation and function.

The casts of earthworms boost soil fertility and help various organisms to inhabit the soil and to complete their life cycle. Macro- and micro nutrients and their cycling process solely depend upon earthworms and it has been shown that macronutrients are abundant around earthworm casts and burrows which supports root growth (Edwards and Bater, 1992). Earthworm casts have been found to contain elevated amounts of NH:, NO;, Mg, K and P relative to the surrounding soil (Lunt and Jacobson, 1944;

Parle, 1963; Gupta and Sakal, 1967; Syers et al., 1979; Syers and Springett, 1984; Tiwari et al., 1989). Earthworms influence the soil structure by forming macropores, which allow oxygen to enter the soil, whereas micropores between the aggregates give a good water-holding capacity (Lavelle, 1988; Willems et al., 1996). In addition to forming macropores and increasing water infiltration, earthworms have been shown to increase soil the aggregate stability (Li and Ghodrati, 1995) and the water-holding capacity (Stockdill, 1982).

Generally organisms are known to avoid areas polluted with contaminants above tolerable levels (Amorim et al., 2005; Fenoglio et al., 2007). Earthworms serve as indicators of soil status such as the level of pollutants e.g. agrochemicals, heavy metals, toxic substances, industrial effluents and human-induced activities e.g. land-management practices and forest degradation (Radha and Natchimuthu, 2010). Any changes in soil properties have great impact on earthworms and hence they serve as indicator organisms for ecotoxicological studies (OECD, 1984).

The human impact on the environment can be scaled by the measurements of heavy metals in the soil, in plants and in animals because metal pollution adversely affects the density and diversity of biotic communities including humans (Bengtsson et al., 1981; Mountouris et al., 2002). the abundance and distribution of earthworm species (*Pontoscolex corethrurus, Perionex excavatus, Dichogaster modigliani* and *Polypheretima elongate*) (Ching et al., 2006), is primarily based on soil the type, soil moisture, texture and the soil constituent. Usually, the abundance and distribution of earthworms in undisturbed soil are greater than those, that soils that have been cultivated, burnt or undergone various crop practices like the use of fertilizers, fallowing etc. Some researchers have focused on the role of earthworm species in their tolerance and absorption of metals, petroleum hydrocarbons and polynuclear aromatic hydrocarbons (PAHs) (Zachary and Reid, 2008; Owojori et al., 2009).

Generally, the potential hazards of various environmental toxicants to soil invertebrates are assessed by bioassays with the keystone species - earthworm. Earthworms are one of the first receptors affected by soil contamination. They are more susceptible to metal pollution than any other groups of soil invertebrates and toxicity data on earthworms are important in determining “Safe levels” for metals and other contaminants in soil.

Heavy metals have been shown to cause lysosomal membrane instability, changes in gene expression, oxidative stress (Spurgeon et al., 2004a; Berthelot et al., 2008; van Gestel et al., 2009), reduced growth (Spurgeon et al., 1994), slower sexual development (Spurgeon and Hopkin, 1996; Spurgeon et al., 2004b), depleted cocoon production, hatchability (Reinecke et al., 2001; Davies et al., 2003; Spurgeon et al., 2004a) and juvenile viability (Bengtsson et al., 1986; van Gestel et al., 1992), increased mortality (Neuhauser et al., 1985; Spurgeon et al., 1994; Davies et al., 2003) and also they affect the population size, abundance and species diversity of earthworms (Spurgeon et al., 2005).

Earthworms, especially the compost worm *Eisenia fetida*, are model organisms for assessing the effects of various chemicals on terrestrial invertebrates (Spurgeon et al., 2003; Nahmani et al., 2007). *Eisenia fetida* (*andrei*), which is a manure species living in organic matter-rich substratum is recommended by the international guidelines (OECD, 1984; ISO, 1998) as a test species and has been mainly used in genotoxicity studies. It has a short life cycle, hatching from cocoons in 3 to 4 weeks, and reaching maturity in seven to eight weeks. It is a prolific species and its rearing in laboratory is simple. It is a representative component of the terrestrial fauna, but is mostly used as a compost worm because of its great potential towards decomposing waste. It is an epigeic species and is sensitivity to soil pollutants compared to other field earthworms.

Leachate is a liquid formed from the percolation of rain water through the disposed wastes. The dissolution of contaminants such as heavy metals in the leachate can pose serious pollution problems. Species richness may be reduced due to metal pollution (Nahmani et al., 2003) and this indirectly alters species interaction (Grzes, 2009). From ecotoxicity point of view, earthworms are likely to be sensitive to tillage, inputs of organic matter and the application of chemicals (Edwards et al., 1995 and Smith et al., 2008). Due to their beneficial role in terrestrial ecosystems, earthworms are often used as an indicator species in ecotoxicological evaluation (Abdul Rida and Bouche, 1995). Previously few attempts have been made to study the effect of industrial discharge on earthworms (Goats and Edwards, 1982; Mishra and Sahu, 1989, 1997; Callahan et al., 1991).

Heavy metal can be defined as any metal with a specific gravity higher than 4.00 and which is toxic and poisonous even at a low concentration (Agbaire and Emoyan, 2012). Heavy metal concentrations in the soil are associated with biological and geochemical activities and are influenced by anthropogenic activities (Agbaire and Emoyan, 2012). Heavy metals are considered serious pollutants because they are toxic and non-degradable (Agbaire and Emoyan, 2012). The accumulation of heavy metals in the soil poses many risks to humans and the ecosystems (Odoh et al., 2011). Most importantly pollution by heavy metals in terrestrial ecosystems is a serious environmental concern due to their non-biodegradability and tendency to accumulate in plants and animal tissues (Otitoloju et al., 2009).

As a by-product phosphogypsum is produced in phosphoric acid plants. Phosphogypsum is generated through a filtration process in phosphoric acid plants where insoluble gypsum is separated from the product i.e. phosphoric acid as efficiently as possible. About 4.5 -5 tons of phosphogypsum as by-product is being generated per ton of phosphoric acid recovered. Phosphogypsum generation in India is about 11 million tons per annum and primarily consists of calcium sulphate dihydrate with small amounts of silica, usually as quartz and un-reacted phosphate rock, radioactive material (like radium, uranium) and heavy metals namely arsenic, cadmium, chromium, mercury, nickel, lead and zinc (HAZWAMS/2012-2013). There was very considerable volume of work and research regarding the positive roles of earthworms in agroecosystems, environmental monitoring and sustainability (Dada et al., 2013) but the concern here is to evaluate the influence of phosphogypsum over various physiological parameters of *Eisenia fetida* under laboratory conditions.

Review of literature

Lee et al. (2009) determined the effect of alkalized phosphogypsum on soil chemical and biological properties. Their study came into the conclusion that by mixing alkalizing phosphogypsum with an alkaline material such as Ca(OH)2 can turn it into a useful material for agricultural utilization. Pure phosphogypsum could damage the soil biological properties.

Mishra et al. (2011) assessed the impact of phosphogypsum amendment on soil physic-chemical properties, microbial load and enzyme activities. They carried out their experiment with 0, 5, 10 and 15% of Phosphogypsum to track the impact and found that the optimal results were recorded in 10%. The experiment summarized with a conclusion that Phosphogypsum amendment will be fruitful at a particular range and can be fatal for the microbial and soil enzyme activities if it exceeded its threshold range.

Chen et al. (2014) investigated the effects of gypsum on trace metals in soil and earthworms. For the study they used FGD (Flue Gas Desulfurization) gypsum which was produced by the removal of SO2 from flue gas streams. The application rates of gypsum ranged from 2.2 Mg ha^{-1} to 20 Mg ha^{-1}. These rates were 2 to 10 times higher than typically recommended. It was observed that the earthworm numbers and biomass were decreased significantly and with the higher rates of gypsum application was found to be hazardous to earthworms and soil integrity.

Rakhimova et al. (2017) evaluated the impact of coal ash and phosphogypsum on soil fertility of Chernozem soils of north Kazakhstan. Soil samples were collected (three times a year) and the initial content of humus, pH, plant nutrient elements and heavy metal content of the soil were tested in laboratory and recorded. The application of Phosphogypsym and coal ashes for fertilization of the soils influenced the growth of soil nutrient elements such as nitrogen, phosphorous, copper and zinc. It also impacted the on neutralization of the soil environment and further investigation is needed to find out actual content of heavy metals (Cu, Zn, Cd and Pb).

Materials and methods

Material

Phosphogypsum

Phosphogypsum was collected from Paradeep Phosphate Limited (PPL), Paradeep, Jgatsinghpur, Odisha, India.

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 0 g%, 4 g%, 8 g% and 10 g% gypsum with 25 g% water.

Method

500 gm 2 mm sieved, air dried soil was taken in polythene packets with moisture maintained at 25 g % by addition of distilled water in all packets. The control consisted of 500 g soil only and PG was added to soil in experimental sets i.e. 4%, 8% and 10%. Ten replicates of each concentration were taken for each parameter (growth, feeding, respiration and regeneration). After moisture addition the packets were left for five days for stabilization. Culture was maintained with 25 g% moisture and 25 °C temperature (Initially we went for 25 g%, 50 g% and 75 g% concentration of phosphogypsum with 25 g% water for the experiment but it was found that worms unable to tolerate such high amount of phosphogypsum percentage and found dead).

Experimental set up for growth

After five days of moisture addition and stabilization of soil, up to 1.70 g of earthworms were inoculated to each packet. Weight of earthworms in each packet was determined and change in weight over initial weight was observed after on 10th, 20th and 30th days. Percentage change in weight of earthworms over zero day culture was estimated.

Experimental set up for feeding

2.5 g of earthworms were inoculated to each packet and estimation of stable aggregates was done at an interval of 10 days by sieving culture sets with water. Carbon content of stable aggregate formed was estimated by Walkley and Black titration method (Walkley and Black, 1934). Then the amount of carbon was converted to energy according to available standard values energy conversion for carbon is 41.44 kJ g^{-1} dry wt. (Remmert, 1980).

Experimental set up for respiration

Out of 10 replica of each concentration, 5 were maintained without earthworms which accounted for microbial respiration. To each packet, an average of 1.5 g of earthworms were added. The respiratory metabolism was estimated on 10th, 20th and 30th day by Alkali Absorption Method (Witkamp, 1966). Carbon dioxide evolution was measured and expressed as mg of $CO_{2.}$ g^{-1} live worm tissue hr^{1} kg^{1} soil.

Experimental set up for regeneration

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 phosphogypsum. The amputed earthworms could not survive in 8% and 10% phosphogypsum. So the regeneration experiment was performed with 4% PG only.

Statistical analysis

Statistical analysis were performed to infer the results (Microsoft excel version 2007). For growth, feeding and respiration, ANOVA test was performed. T-test was performed for regeneration. Comparisons of values were made and values at $p \leq 0.01$ are said to be significant.

Results

Growth

Table 1 and *Figure 1* indicate the changes in the growth i.e. changes in weight pattern of *Eisenia fetida* earthworm under the impact of phosphogypsum in laboratory cultures. In the first phase of estimation (initial 10 days), a positive increase of about 1.53% in weight in control set ups was observed in comparison to experimental set ups. On exposure to 4%, 8%, and 10% phosphogypsum, there was significant decrease (at 0.01 level) of about 23.07%, 23.07% and 75% respectively over zero day. At 20 days, the weight percentage reached up to 5.03 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 phosphogypsum. After the end of final phase (last 10 days), 96.40% decrease in weight over initial weight at 10% phosphogypsum. In at 4% and 8%, weight was decreased by 30.93 and 74.82% over initial weight respectively. But in Control sets, there was a remarkable growth noticed (21.53%). Analysis of variance showed significant impact of phosphogypsum treatment on earthworm weight at 0.01 level of significance.

Table 1. *Weight of Eisenia fetida earthworm under impact of phosphogypsum 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

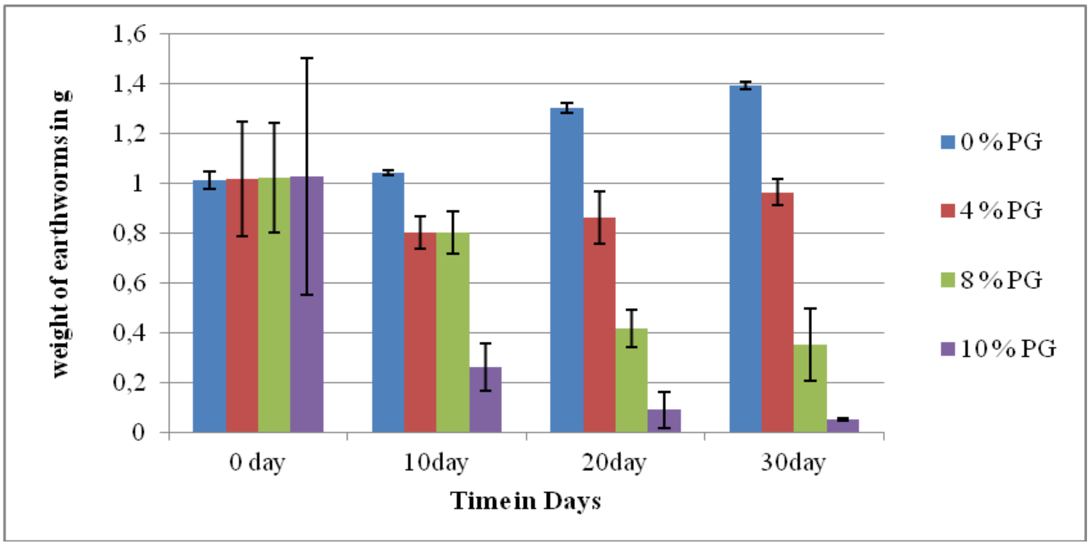

Figure 1. *Change in weight of Eisenia fetida earthworm under the impact of phosphogypsum in laboratory culture. (Mean ± standard deviation)*

Feeding

After 10 days of exposure to 0, 4, 8, 10% of phosphogypsum, the weight of stable aggregates formed was 12.67, 11.72, 12.40 and 9.42 g g^{-1} live tissue (*Fig. 2; Table 2*) and a visible decrease of about 0.009%, 97.66% and 99.20% in stable aggregate formation as comparison to zero day in 4, 8 and 10% of phosphogypsum respectively (*Fig. 3*). On completion of 30 days there was decrease of about 99.7%, 99.8% and

99.93% respectively was observed (*Table 2*). When it comes to the matter of carbon content of stable aggregate formed by the earthworms, a consistent and significant decline was noticed by the increase of concentration dose and exposure duration (*Fig. 4*). Highest energy content of stable aggregate formed was 784.74 kJ kg^{-1} soil g^{-1} live tissue at 0% and lowest was 349.09 kJ, kg^{-1} soil, g^{-1} live tissue at 10% on 30th day. A remarkable significance difference noticed in stable aggregate formed at the end of 30 days in comparison to 10th day. After 30 days at 0%, the weight of stable aggregate formed by the earthworms was 32.65 g g^{-1} live tissue which was much higher when it compared to 4, 8 and 10% (22.45, 18.16 and 16.20 g g^{-1} live tissue respectively). Analysis of variance showed significant decrease in the stable aggregate formation by the worms (at 0.01 level of significance) on application of phosphogypsum. Significant decrease in energy content of stable aggregate was also found at 0.01 level of significance.

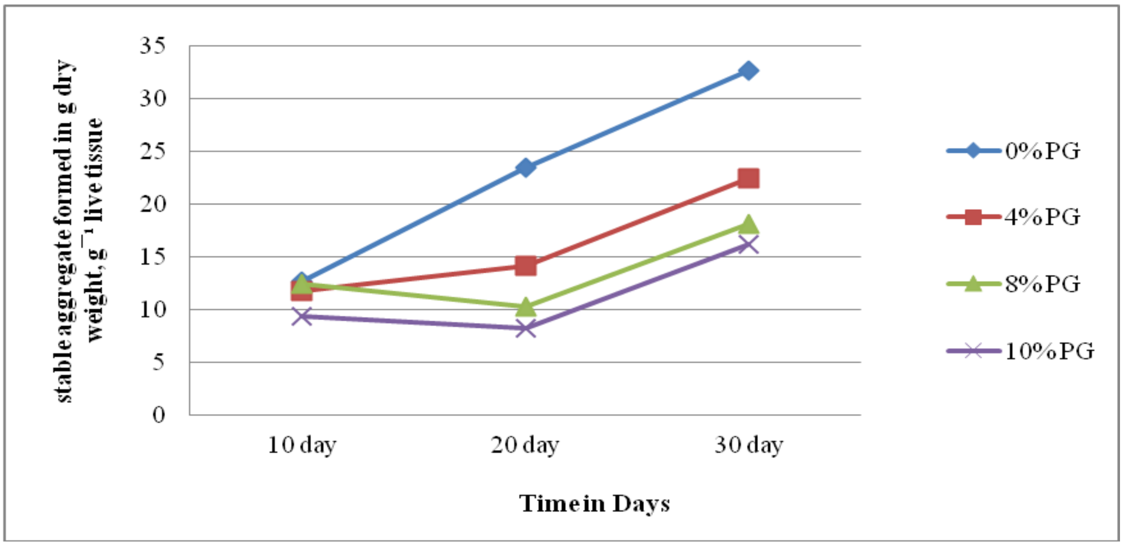

***Figure 2.** Stable aggregate formed by Earthworm Eisenia fetida under the impact of phosphogypsum in laboratory condition*

***Table 2.** Stable aggregate formed by Eisenia fetida under the impact of phosphogypsum*

Culture time in days	Parameters analysed	Concentration of phosphogypsum in percentage			
		0	4	8	10
10	Weight of stable aggregate	12.67	11.72	12.40	9.42
	% change over “0” percent	0	-0.009	-97.66	-99.20
	Energy content	283.52	237.98	226.09	160.04
20	Weight stable aggregate	23.40	14.16	10.32	8.25
	% change over “0” percent	0	-99.12	-99.57	-97.81
	Energy content	552.72	332.73	209.55	164.44
30	Weight stable aggregate	32.65	22.45	18.16	16.20
	% change over “0” percent	0	-99.7	-99.8	-99.93
	Energy content	784.74	530.28	414.65	349.09

Weight: g dry weight stable aggregates, g^{-1} live tissue
% change: gain or loss
Energy content: kJ kg soil g^{-1} live tissue

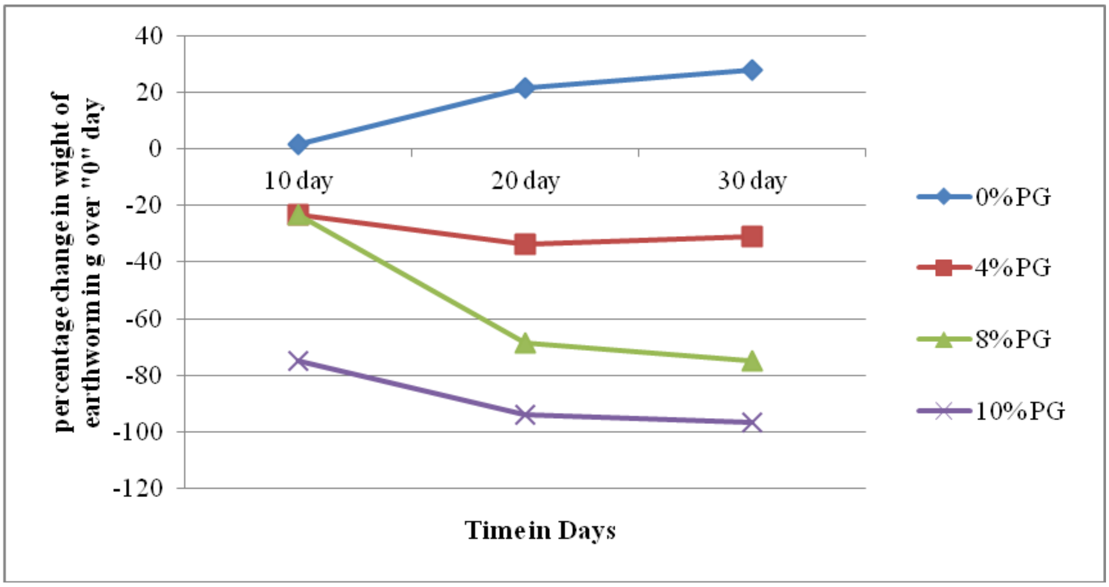

Figure 3. *Percentage change in weight of earthworm Eisenia fetida under the impact of phosphogypsum in laboratory cultures*

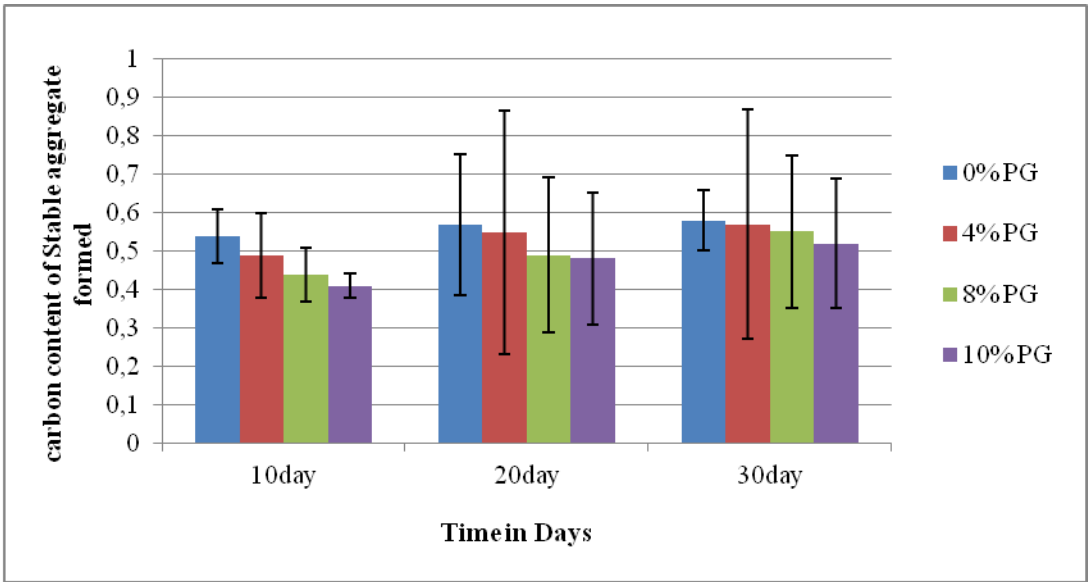

Figure 4. *Carbon content of stable aggregate formed by earthworm Eisenia fetida under the impact of phosphogypsum in laboratory cultures. (Mean ± standard deviation)*

Respiration

The rate of respiration was found to increase with duration and treatment. After 10 days of exposure the rate of respiration was found to be 0.0564, 0.0565, 0.0567 and 0.0568 mg of CO_2 evolved, g^{-1} live worm tissue hr^{-1} kg^{-1} soil at 0%, 4%, 8% and 10% of phophogypsum, respectively (*Fig. 5; Table 3*). At the end of the last phase (30 days), there was increase of about 0.17%, 0.34% and 0.52% in rate of respiration on exposure to 4%, 8% and 10% phosphogypsum over control respectively. The mg of CO_2 i.e. evolved, g^{-1} live tissue hr^{-1} kg^{-1} soil increased from lower concentrations to higher concentrations of phosphogypsum as the days increased. Analysis of variance showed significant increase in respiratory metabolism of earthworm at 0.01 level of significance under the impact of phosphogypsum.

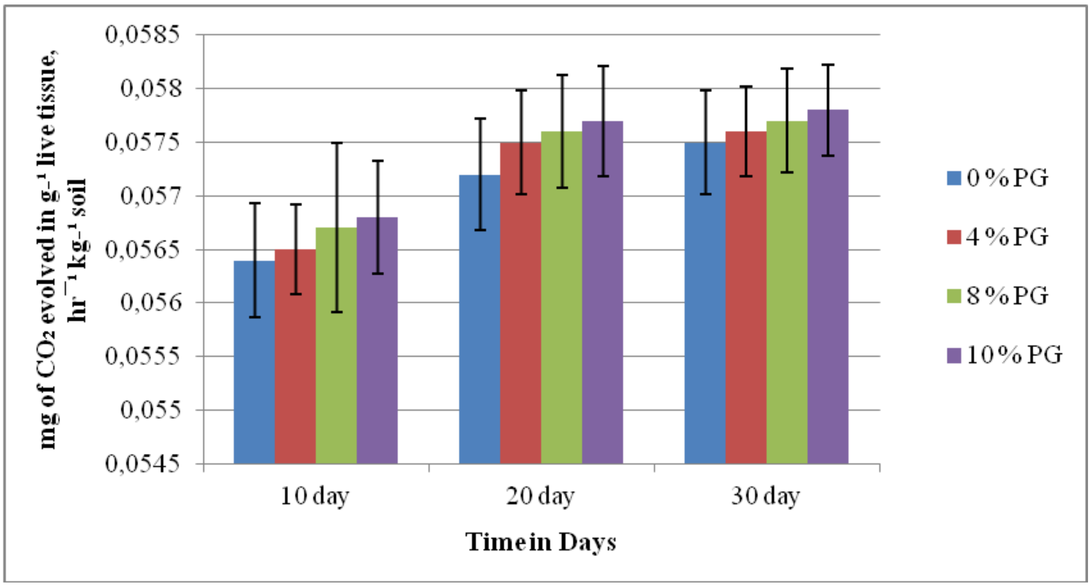

Figure 5. *Respiratory metabolic rate of earthworm Eisenia fetida under the impact of phosphogypsum. (Mean ± standard deviation)*

Table 3. *Respiratory metabolic rate of Eisenia fetida earthworm under the impact of phosphogypsum*

Culture time (in days)	**Rate of respiration (mg of CO_2 evolved, g^{-1} live worm tissue hr^{-1} kg^{-1} soil) at different concentrations of phosphogypsum in percentage**			
	0%	**4%**	**8%**	**10%**
0-10	0.0564 ± 0.000516	0.0565 ± 0.000527	0.0567 ± 0.000527	0.0568 ± 0.000422
10-20	0.0572 ± 0.000789	0.0575 ± 0.000527	0.0576 ± 0.000516	0.0577 ± 0.000483
20-30	0.0575 ± 0.000527	0.0576 ± 0.000516	0.0577 ± 0.000483	0.0578 ± 0.000422

Mean ± standard deviation

Regeneration

The amputated earthworms could not survive beyond two days in 8% and 10% phosphogypsum. So the experiment was continued with control and 4% phosphogypsum. On completion of 10 days of exposure to 0% and 4% of phosphogypsum, the percentage increase in regenerated segments was found to be 6.28% and 2.68% respectively (*Figs. 6* and *Table 4*). After 20 days of exposure to 0% and 4%, the percentage of regenerated segments was found to be 10.64 and 5.72% respectively. At the end of 30 days, the percentage increase in regenerated segments was found to be 13.4% in 0% and 8.4% in 4%. The regenerated parts of the worm grown in 4 % phosphogypsum can be marked in the *Fig. 7* after the completion of 30 days. T-test reveals that impact of phosphogypsum has a significant effect on *Eisenia fetida* at 0.01 level of significance.

DOI: http://dx.doi.org/10.15666/aeer/1604_44554476

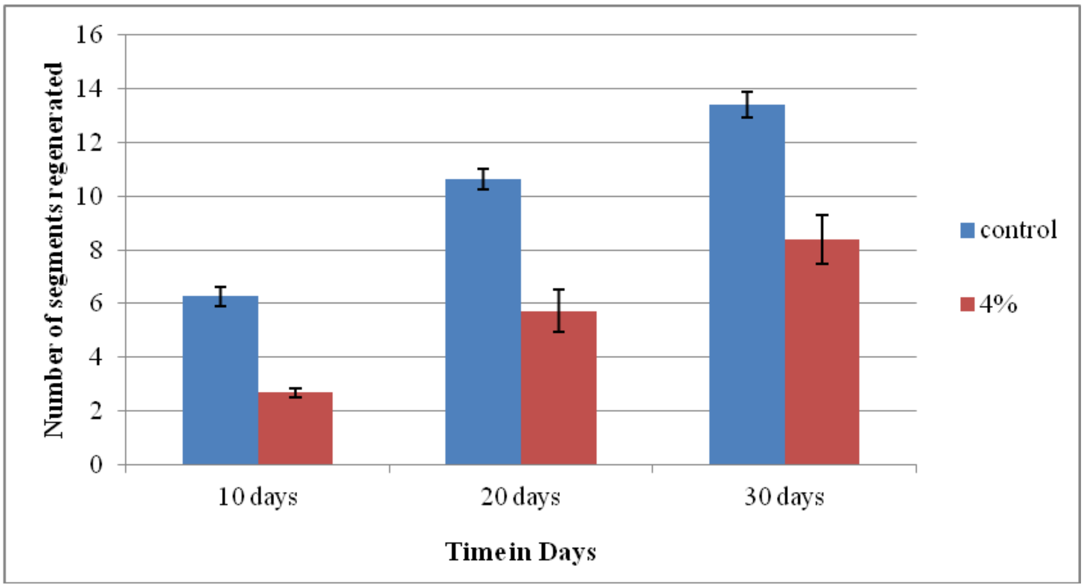

Figure 6. *Percentage change in regeneration of earthworm Eisenia fetida under the impact of phosphogypsum in laboratory culture. (Mean ± standard deviation)*

Figure 7. *Regenerated segments of earthworm Eisenia fetida at 4% phosphogypsum*

Table 4. *Regeneration of Eisenia fetida earthworm under the impact of phosphogypsum in laboratory culture*

Culture in days	Parameters analysed	Conc. of PG 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

DOI: http://dx.doi.org/10.15666/aeer/1604_44554476

Discussion

Earthworms ingest organic matter of the soil particle and excrete this in the form of casts, which get deposited on the soil surface and this cast acts as a natural fertiliser for the soil and boosts the soil fertility. Loss of fertility is directly proportional to stable aggregate formed by earthworms. Heavy metallic factor present in the soil directly affects the various physiological activities of *E. fetida*. Majority of heavy metals are toxic to living organisms when retained in the soil and interfere with the biochemical processes and alter the ecological balance (Nwuche and Ugoji, 2008). These heavy metals do not biodegrade rather they bioaccumulate and this may have adverse effects on the biodiversity of the area if the organisms do not develop a mechanism of adaptation to it. Nickel and lead accumulated significantly in worm bodies during the 3-weeks exposure whereas zinc accumulation was efficiently regulated. Nevertheless zinc exposure significantly inhibited the weight gain. Inhibition of weight gain in zinc-exposed worms may be putatively explained by the high energetic costs of efficient zinc regulation (Podolak et al., 2011).

In many other case studies, *D. veneta* (Kwadrans et al., 2008) and *Aporrectodea caliginosa* (Dutkiewicz et al., 2009) were maintained for 4 and 8 weeks, respectively, in soil samples soaked with Cd, Cu, Pb, or Ni chlorides. Body weights of *D. veneta* were unaffected by 4-weeks metal exposure. In a study by Spurgeon et al. (1992), control worms and those on the lowest concentration of metals had slightly increased in weight after 1 week. However, in subsequent weeks their weight declined. Malecki et al. (1982) tested the effect of Cd, Cu, Pb, Ni and Zn on the physiological activity of *Eisenia fetida* in laboratory cultures. They concluded that cadmium was the most toxic metal, as significant decreases in growth noticed. Here it is important to mention that Cadmium is one of the heavy metal present in the phosphogypsum beyond the recommended range. It was observed (Khalil, 2013) that earthworms *Allolobophora caliginosa* feed less and *Pheretima hawayana* escaped into their burrows when exposed to arsenate. Miguel et al. (2012) revealed in a study that weight and mortality of the worms were significantly affected by high levels of heavy metals.

Metal exposure can imbalance the host-bacteria relationship, as evidenced in *D. veneta* after 3-days exposure to filter paper soaked with water (controls) or metal (Zn, Cu, or Cd) chlorides (Podolak et al., 2011). It may assumed that effects of metal exposure on immunity are rather associated with the disrupted balance between the worm immune system and microbial impact from surrounding metal-polluted soil (Salice and Roesijadi, 2002; Wieczorek-Olchawa et al., 2003; Olchawa et al., 2006). Various results indicated that earthworm activity increases the mobility and bioavailability of heavy metals in soil (Wen et al., 2004; Sizmur and Hodson, 2009). But the matter is how far and how much worms can accumulate and able to rotate the mobility of heavy metals. Although concentrated heavy metals in the earthworm's body demonstrate the ability of *E. fetida* to accumulate the heavy metals in their body still it needs to reveal the limit or range which is non-deleterious.

Fordsmand et al. (1998) investigated the toxic effects of nickel on survival, growth, and reproduction of *Eisenia veneta* for 4 weeks of exposure to a nickel chloride spiked loamy sand soil. Nickel caused a significant toxic effect on *E. veneta* at soil concentrations above 85 mg Ni/kg. Survival of adults was only reduced at concentrations above 245 mg Ni/kg, while adult and cocoon wet weight were not affected by soil nickel concentrations up to 700 mg Ni/kg. The results of our study are in close agreement with Ma (1983), Neuhauser et al. (1985), Bengtsson et al. (1986), Gestel and Dis (1988), Gestel et al. (1989, 1991, 1992). The results of the present study clearly correlate with the work of Zaltauskaite (2010) who reported that *E. fetida* exposed to different concentrations of lead in the soil tended to lose more weight than those in control.

Single metal such as Cu can affect the physiology and growth of *E. fetida* may hampered due to increased tissue concentration of Cu., while the cocoon hatchability and number of juveniles increased as the tissue Cu concentration increased (Leduc et al., 2008). So it was a combine impact of heavy metals which might be more hazardous and has more pronounced toxicity than the individual metals (Olaniya et al., 1991). In some situations *E. fetida* can allocate more energy to growth than reproduction, delaying other physiological development (Leduc et al., 2008). A combination of As, Cr and Cu can impair growth, affect reproduction and cause death also (Leduc et al., 2008).

Sivakumar and Subbhuraam (2005) evaluated the toxic effects of chromium exposure on *E. fetida* and found 14-day LC50 values ranging from 1656 to 1902 mg kg^{-1} for Cr (III) and from 222 to 257 mg kg^{-1} for Cr (VI) in ten soils. Cocoon production by *E. fetida* was reduced by 50% after exposure to a range between 679 and 1110 mg Cr (III) kg^{-1} (Lock and Janssen, 2002b). In a contradictory study Shahmansowri et al. (2005) revealed that though heavy metals such as Cr, Cd, Pb, Cu and Zn were bioaccumulated by *E. fetida,* but a significant reduction of body weight only marked when exposed to higher concentration. Similarly pattern of findings also observed in weight *Pheretima guillelmi* at high concentration of lead in (Rongquan and Canyang, 2009). Among *E. albidus, E. crypticus* and *E. fetida* the most sensitive species towards industrial waste material was *E. fetida* (Kobeticova et al., 2010). The weight of worms was significantly affected by high levels of heavy metals in the study of Miguel et al. (2012).

Impact can vary due to the different contact times during which the earthworm exposed to contaminants (Haghparast; 2013; Golchin et al., 2013). Concentration of the metals in the body of earthworms can reach the toxic level if the contact time of earthworm is a prolonged duration (Hagparast et al., 2013). Jamshidi and Golchin (2013) also confirmed same. Thus, the potential effects of metals may be overlooked or underestimated in short term experiments (Bengtsson et al., 1986), which may explain the truth that sometimes despite the high levels of metal contamination in the soil, no significant impact may not noticed like Kennett et al. (2002). In a such kind of study, Honsi et al. (2003) reported no effect of heavy metals (Cu, Zn, Cd and Pb) on survival of earthworm *E. fetida* exposed to contaminated soils in Norway despite the fact that the concentrations of metals were high (max Pb – 8750 µg g^{-1}, Cd – 110 µg g^{-1} soil). Kaur and Hundal (2016) observed a very sound impact of heavy metals in the body weight of earthworms. Berthelot et al. (2008), Matuseviciute et al. (2005) and van Gestel et al. (2009) findings also support our results. Spurgeon and Hopkin (1996) also argued that the worms living in metal polluted soils reached the lower weight or needed more time to reach the maximum weight than in non-polluted sites.

Trace elements added to the soil with gypsum or any soil amendment may be concentrated in food chains as the elements are consumed and passed from one trophic (feeding) level to another level (Duffy and Gulledge, 2011). Garg et al. (2009) found a pattern of heavy metals accumulation in *E. fetida* after 45 days i.e. Cr>Cd>Pb>Zn and Cd had the greatest detrimental effect on cocoon production and matter of concern is these heavy metals are one of the major part of phosphogypsum.

During their study, Avila et al. (2009) identified that increasing the organic material can reduce the toxicity of heavy metals in the body of earthworms. Irizar et al. (2015) concluded during their study that, if the organic material in the soil is low, earthworms are not able to digest the soil and, as a result, the toxicity of cadmium increases in them, and the mortality and disorder in reproduction rise. Haghparast et al. (2013) showed that organic material is a source of energy for *Eisnia Fetida* earthworms and increases the percentage of their survival. Avila et al. (2009) showed that adding 5% of organic matter to soil contaminated with chromium at a concentration of 0.06 mg/g after 21 days gave no bioremediation, but after 42 days the efficiency of bioremediation (18.33%) increased. At the concentration of 0.1 mg/g the bioremediation efficiency of 30% after 21 days reached 53% after 42 days.

Heavy metallic cations are said to be more mobile or dynamic under acid conditions (Alloway, 1996) as the correlation between soil pH and micronutrients availability has been one of the major aspect of soil (Brady, 1990; Joshi et al., 1983, Sharma et al., 2003; Akinrinde et al., 2005). Although organisms like earthworm can accumulate a high concentration of heavy metals in the body (Shahmansouri et al., 2005; Li et al., 2010; Brewer and Barrett, 1995; Bamgbose et al., 2000) but mineralization of dead earthworms releases accumulated heavy metals back to the soil (Morgan and Morgan, 1988a). The amount of metals accumulated within earthworm tissues is partly dependent on the absolute concentration of metal within a given soil and physiochemical interactions (Ma et al., 1983). When a contaminant is not in equilibrium with the soil, it becomes more bioavailable to living organisms (Davies et al., 2003). Phosphogypsum is one of the industrial waste materials which is acidic in nature and contains various heavy metals. Another major concern here is *E. fetida* has already been reported as very sensitive species towards industrial waste material exposure by Kobeticova et al. (2010).

Earthworms are well capable of regenerating lost segments, but different species have different regeneration ability. After studying regeneration in different kinds of species for 20 years, Gates (1949) published some of his findings that show for certain species there is a possibility of growing into two intact ones from a bisected specimen. *Eisenia fetida* with head regeneration, in an anterior direction, possible at each inter-segmental level back to and including 23/24, while tails were regenerated at any levels behind 20/21, i.e., two worms may grow from one.

Three potential pathways exist for the removal of chemicals from tissues; elements can be regulated by excretion from the body, bound within the matrix of inorganic granules and attached to proteins or other ligands (Tessier et al., 1994). If metals are detoxified primarily by excretion, body concentrations should decrease when previously exposed individuals were transferred to a clean environment. However, if an element is bound in an inorganic matrix or to organic ligands, metal levels may remain constant, even after the exposure has ceased (Spurgeon and Hopkin, 1999). Animals exposed to high concentrations of metals over a long period, the storage capacity of the hepatopancreas may become saturated, allowing metals to pass into the haemolymph and interfere with sensitive biochemical processes (Spurgeon and Hopkin, 1999).

Heavy metals are strongly bound to soils rich in organic matter or clay (Sauve, 2002) and simultaneously earthworm developed specific trafficking, storage pathways and redistribution capacity to regulate the heavy metals, especially essential trace metals such as Cu and Zn, in their bodies that may lead to balance between uptake and excretion (Dallinger, 1993; Morgan and Morgan, 1999). The regulatory capacity of metals can partly explain the ability of some earthworm species, like *D. octaedra*, to live even in a highly metal contaminated areas. Thus, the metal regulation may also have contributed to the development of metal resistance observed in some of the earthworm populations (Bengtsson et al., 1992; Langdon et al., 2001a, b; Reinecke et al., 1999). One additional factor that may reduce harmful effects of metals on the earthworms and also increase intraspecific variation in body burdens is heterogeneous distribution of metals in the soil (Lukkari et al., 2004).

Conclusion

Earthworms form the base of various food chains because they are preyed on by many species of snakes, mammals, and invertebrates (Edwards and Bohlen, 1996) and could increase biomagnification in various trophic levels of the food chain. This whole concept can upset the food chain as well as the food web. Increasing pollution and spontaneous addition of pollutant to soil through anthropogenic sources can alter growth, feeding, regeneration and respiration of *E. fetida* by both direct and indirect effects. These physiological activities of worm can be impaired by direct toxic effects of metals or by bringing changes in the energy budget as an individual attempts to prevent accumulation in sensitive tissues. In earthworms, cadmium, lead, and some zinc are detoxified by binding in granules (chloragocytes) or metallothionein like proteins in the chloragogenous tissue (Morgan and Morgan, 1988b; Morgan et al., 1989). In contrast, copper and the remaining zinc are eliminated by an excretion mechanism (Morgan and Morgan, 1990, 1991). The mechanisms of metal sequestration and elimination have metabolic costs in both development of the system and for the maintenance and repair. This increased the requirement for maintenance energy will ultimately result in a reduction in the energy available for growth and development (Donker et al., 1993a). If there is an increased energy demand for metal sequestration and elimination, this will decrease the energy available for other physiological activity and ultimately affects the organism. Present findings very much evident and transparent about the sensitivity of the worm *E. fetida* towards metallic contamination and indicates juvenile *E. fetida* is more sensitive to metal-contaminated soils than that of mature worms (Spurgeon and Hopkin, 1996). After 4-5 weeks of culture significant reduction was marked in the weight, feeding activity and regeneration capacity of the worm. In contradiction, rate of respiration clearly increases by the increasing of exposure duration with concentration dose. Number of studies on other soil dwelling invertebrates also suggests a clear reduction in growth on exposure to metal-contaminated diets due to avoidance of contaminated food (Drobne and Hopkin, 1995; Laskowski and Hopkin, 1996). In contradiction to our study Podolak et al. (2011) stated that metals may be either regulated (Zn) or accumulated (Ni, Pb) in worm bodies, with or without deleterious effects on body weights, immune competent cells and physiological activity putatively due to their differential impact on soil and coelom-inhabiting microbes. For last few years, phosphogypsum is being used in agricultural soil as a calcium supplement to enhance the crop production (Nayak et al., 2011). Mullino and Mitchell (1990) have reported the use of phosphogypsum to increase yield and quality of forages in Florida. But respiration of soil was deeply affected by phosphogypsum application (Delaune et al., 2006) which directly hampers the physiological activities of soil dwelling organisms including earthworms. However, the chemical and biological response of soil to the waste amendment needs to be thoroughly investigated before recommending its large scale field application. When it comes to statistical part to verify the significance of phosphogypsum on *Eisenia fetida* (at 0.01 significance level), it was clearly significant. All the physiological activity of the earthworm was significantly hampered due to phosphogypsum and it was almost lethal. The heavy metallic constituents of phosphogypsum adversely affect the growth, feeding, respiration and regeneration activity of the worm.

Nevertheless earthworm based assessment is complicated by the fact that earthworms can develop tolerance to various pollutants, as documented in several studies of populations that have been in contact with high polluting sources over long periods (Bengtsson et al., 1992; Morgan and Morgan, 1992). The direct measurement of heavy metal concentrations in earthworm tissues could provide a means of assessing environmental pollution levels, given the demonstrated correlation between soil contamination and earthworm metal bioaccumulation (Motalib et al., 1997). But here the matter of concern is to generalize these vital facts regarding impact of heavy metals upon soil macrofauna in particular earthworms and standardize the accumulation range. How much and how far earthworms are able to tolerate such wide range and hazardous impact of heavy metals with varying composition of soil. This is a big question to answer. It becomes more necessary to generalize these facts and must be much more aware and watchful towards soil contamination.

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