

under controlled conditions. Adult and mature earthworms, which possessed clitellae and having an individual wet weight of 200 ± 30 mg (after excreting the gut content) were selected for testing and further experiment [19].

Used chemicals and test solution preparation

The commonly used chemical fertilizer urea was used as testing chemical. It was purchased from the regular fertilizer distributor of Balasore district. Aqueous solutions of various concentrations were prepared by dissolving the urea in deionized water (distilled water) [20,21]. The concentrations were prepared in 5 mg/ml, and the toxicity was measured as $\mu\text{g}/\text{cm}^2$.

Acute toxicity testing

An acute toxicity testing of urea was performed on earthworms using a simple paper contact method projected by OECD testing guideline No. 207 [22,23]. This is a plain screening test to identify the toxic potential of the chemical fertilizer to earthworm. The protocol test vial was a plastic Petri dish of 14 cm in diameter and 2 cm in height. Round filter paper (Whatman No. 1 filter paper) of size 8.5 cm diameter was cut and resized to the suitable size and placed in such a way that sides are lined with the filter paper. Five regular concentrations were prepared by dissolving urea in 5 ml deionized (distilled) water in a general geometric series (5, 10, 20, 40, and 80 mg) and were pipette into each test vial to wet the filter paper bed. Blank control tests were performed with the 5 ml of deionized water only. For each treatment, 10 replicates of experimental series were used, each consisting of single earthworm per test vial. Adult and mature earthworms, which possessed clitellum and having an individual wet weight of 250–350 mg, were selected for testing and experiments. Earthworms were washed briefly with deionized water and were kept on moist filter paper for 3 h to get excrete the gut content, after which it was rinsed again with deionized water regularly, blotted on the filter paper, and placed in a testing vial (one earthworm per vial). After the introduction of earthworm, the vial was covered with plastic film that had been punched with small holes using needles for the respiration and air accumulation purposes. Tests were done in the darkroom at $28 \pm 20^\circ\text{C}$ for 48 h. In a regular time interval of 1 h, the earthworm was monitored for mortality by a gentle mechanical stimulus to the front part of the body.

RESULTS AND DISCUSSION

The lethal toxic concentration of urea to *E. fetida* was evaluated as $28 \mu\text{g}/\text{cm}^2$ by an average calculation. Thus, the comparative toxicity grade of urea was categorized as "very toxic" to *E. fetida*. With consistent area of contact exposure of urea to the earthworm in different concentrations showed various toxic effects in filter paper substrate medium on the Petri plate bed. A geometric concentration series of test solution (5, 10, 20, 40, and 80 mg/5 ml) was prepared and tested, in which mortality of earthworms was observed in 20, 40, and 80 mg concentrations after 18, 12, and 6 h, respectively (Table 1). Only the 5 mg/5 ml concentration was non-toxic to earthworm for 48 h. The lethal concentration for 48 h to *E. fetida* was 10 mg/5 ml concentration (Fig. 2a). The deleterious effects of urea on earthworm were lesions, inflammations, and disjuncting of the posterior body parts (Fig. 2b). The earthworm was not able to move around after 40 h, neural retention and defoliation starts, and the body parts separate leading to the death.

As the neural degradation and the body part deformation are occurred, the filter paper bed was absorbed with the body fluid and serum of the earthworms. Hence, the lethal effect of the regularly used chemical fertilizer urea is killing the friend of farmer in a drastic way.

Contact filter paper testing is an original screening technique protocol to evaluate the comparative toxicity of chemicals and fertilizers to the earthworms. In this preliminary screening experiment, the chemicals are shocked or absorbed into the earthworm body mainly through the outer dermal layer of skin when it is moving around the filter paper [24-26]. Although it fails to signify the condition in soil, it is more significant to know the toxic grade of a particular chemical, whether it

is toxic or non-toxic. If the chemical proved to be toxic, further extensive study on synthetic soil can be carried out and performed. If the chemical proved to be non-toxic, there is no need for the comprehensive study [27]. It has been demonstrated for many decades that most of the inorganic mineral fertilizers are non-toxic to earthworms; however, it has been disproved in this systematic study.

Statistical analysis

For the filter paper contact method, the toxicity is expressed basically in $\mu\text{g}/\text{cm}^2$. Based on the resulting 48-h LC50 values lethal dose [28,29], the urea fertilizer will be classified as super toxic element ($<1.0 \mu\text{g}/\text{cm}^2$), extremely toxic at $1-10 \mu\text{g}/\text{cm}^2$, very toxic at $10-100 \mu\text{g}/\text{cm}^2$, moderately toxic at $100-1000 \mu\text{g}/\text{cm}^2$, or relatively non-toxic at $>1000 \mu\text{g}/\text{cm}^2$. Moreover, the lethal time duration for the individual earthworms per the 5 ml solution testing is determined and is graphically represented (Graph 1).



Fig. 1: The accumulated and adopted to the artificial soil bed

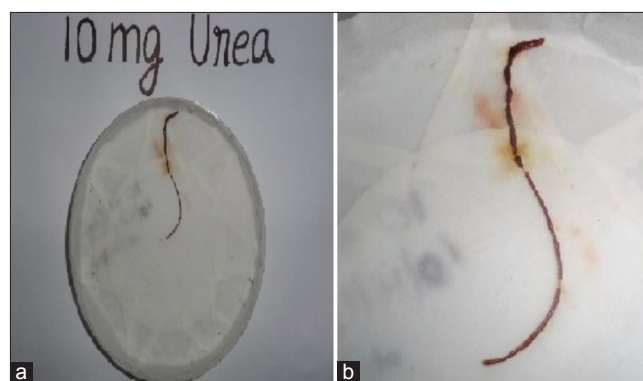
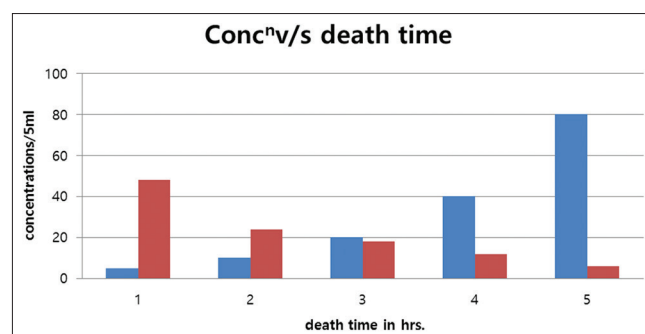


Fig. 2: (a) Mortality of *Eisenia fetida* at 10 mg/5 ml treatment of urea. (b): Degradation effects such as lesions, inflammations, and separation of body parts observed on *E. fetida*



Graph 1: The death time and concentration ratio

Table 1: The death time period per different concentrations

Concentrations	5 mg/5 ml	10 mg/5 ml	20 mg/5 ml	40 mg/5 ml	80 mg/5 ml
Death hours	48	24	18	12	6

CONCLUSION

From the above results, it may be concluded that the acute toxicity of urea on *E. fetida* using a simple paper contact method was actually significant in confirming the toxic impending. The application of environmentally levelheaded doses of urea exposed the possible harmful effects on earthworms when comes in contact directly. Thus, in future, this method will be necessary to find an approach to establish the sensitivity of the earthworm's acute toxicity before going for the evaluation in soil (i.e., acute and chronic toxicity tests in artificial soil sample). Soil being a very complex system, it is often difficult to compare toxicity information directly. Although the paper contact method ignores the contribution of soil compositions, it has a high advantage of reproducibility and the probable of direct comparison of results. Using the data obtained from this method, the assortment of test concentration for acute and chronic toxicity tests can also be indomitable. Thus, this work fulfills the objective that the soil health and its predator's effect. The necessary precautions and regulations should be implemented for the usage of the chemical fertilizers like urea on the agricultural lands. The social awareness is most needed for this serious issue about the soil health.

REFERENCES

- Bartlett MD, Briones MJ, Neilson R, Schmidt O, Spurgeon D, Creamer RE. A critical review of current methods in earthworm ecology: From individuals to populations. *Eur J Soil Biol* 2010;46:67-73.
- Karanjkar AS, Naik RL. Acute toxicity: Novel mode of pesticides on earthworm. *Int J Plant Prot* 2010;2:182-5.
- Ma WC, Brussaard L, de Ridder JA. Long-term effects of nitrogenous fertilizers on grassland earthworms (*Oligochaeta: Lumbricidae*): Their relation to soil acidification. *Agric Ecosyst Environ* 1990;30:71-80.
- Miyazaki A, Amano T, Saito H, Nakano Y. Acute toxicity of chlorophenols to earthworms using a simple paper contact method and comparison with toxicities to fresh water organisms. *Chemosphere* 2002;47:65-9.
- OECD. Organization for Economical Cooperation and Development Guideline for Testing of Chemicals, No.C207, Earthworm Acute Toxicity. Paris, France: OECD; 1984.
- Roberts BL, Dorough HW. Relative toxicities of chemicals to the earthworm *E. fetida*. *Environ Toxicol Chem* 1984;3:67-78.
- Velki M, Hackenberger BK, Abbiramy KS, Ross PR. Biomarker responses in earthworm *E. fetida* to pirimiphos-methyl and deltamethrin using different toxicity tests. *Chemosphere* 2013;90:1216-1226.
- Wang Y, Cang T, Zhao X, Yu R, Chen L, Wu C, et al. Comparative acute toxicity of twenty-four insecticides to earthworm, *Eisenia fetida*. *Ecotoxicol Environ Saf* 2012;79:122-8.
- Xiao H, Zhou QX, Liang JD. Single and joint effects of acetochlor and urea on earthworm *Eisenia foelide* populations in phaozem. *Environ Geochem Health* 2004;26:277-83.
- Lal R, De Vleeschauer D. Influence of tillage methods and fertilizer application on chemical properties of worm casting in a tropical soils. *Soil Tillage Res* 1982;2:37-52.
- Werner MR, Dindal DL. Earthworm community dynamics in conventional and low-input agroecosystems. *Rev Ecol Biol Sol* 1989;26:427-37.
- Lofs-Holmin A. Influence of agricultural practices on earthworms (*Lumbricidae*). *Acta Agric Scand* 1983;33:225-34.
- Edwards CA, Loftly JR. Nitrogenous fertilizers and earthworm populations in agricultural soils. *Soil Biol Biochem* 1982;14:515-21.
- Tiwari SC. Effects of organic manure and NPK fertilization on earthworm activity in an oxisol. *Biol Fertil Soils* 1993;16:293-5.
- Rathore DS. Use of Domestic and Industrial Waste Water for Vermiculture and Soil Fertility. Ph.D. Thesis, MLS University, Udaipur; 2011.
- Bremner JM. Recent research on problems in the use of urea as a nitrogen fertilizer. *Fert Res* 1995;42:321-9.
- Lalthanzara H, Ramanujam SN. Effect of fertilizer (NPK) on earthworm population in the agroforestry system of Mizoram, India. *Sci Vision* 2010;10:159-67.
- Bunemann EK, Schwenke GD, Zwieten LV. Impact of agricultural inputs on soil organisms. *Austral J Soil Res* 2006;44:379-406.
- Iordache M, Borza I. Relation between chemical indices of soil and earthworm abundance under chemical fertilization. *Plant Soil Environ* 2010;56:401-7.
- Yang X, Warren M, Xiaoming Z. Fertilization responses of soil litter fauna and litter quantity, quality, and turnover in low and high elevation forests of Puerto Rico. *Appl Soil Ecol* 2007;37:63-71.
- Tran TS, Dayegamiye A. Long-term effects of fertilizers and manure application on the forms and availability of soil phosphorus. *Can J Soil Sci* 1995;75:281-5.
- Bilalis D, Sidiras N, Vavoulidou E, Konstantas A. Earthworm populations as affected by crop practices on clay loam soil in a Mediterranean climate. *Acta Agric Scand Sect B Plant Soil Sci* 2009;59:440-6.
- Mathews BW, Carpenter JR, Sollenberger LE, Hisashima KD. Macronutrient, soil organic carbon, and earthworm distribution in subtropical pastures on an andisol with and without long-term fertilization. *Commun Soil Sci Plant Anal* 2001;32:209-30.
- Smetak KM, Johnson-Maynard JL, Lloyd JE. Earthworm population density and diversity in different-aged urban systems. *Appl Soil Ecol* 2007;37:161-8.
- Estevez B, NEstevez B, A, Coderre D. The effect on earthworm abundance and selected soil properties after 14 he es of solid cattle manure and NPKMg fertilizer application. *Can J Soil Sci* 1996;76:351-5.
- Potter DA, Bridges BL, Gordon FC. Effect of N fertilization on earthworm and micro arthropod populations in Kentucky bluegrass. *Turf Agron J* 1985;77:361-12.
- Scullion J, Ramshaw GA. Effects of various manurial treatments on earthworm activity in grasslands. *Biol Agric Hort* 1987;4:271-81.
- Jen-Hshuan C. The Combined use of Chemical and Organic Fertilizers and/or Biofertilizers for Crop Growth and Soil Fertility. Bangkok 10900 Thailand: International Workshop on Sustained Management of the Soil-Rhizosphere System for Efficient Crop Production and Fertilizer use Land Development Department; 2006.
- Qi-xing Z, Qian-ru Z, Dong LJ. Toxic effects of acetachlor and methamidophos on earthworm *Eisenia fetida* in phaozem, northeast China. *J Environ Sci* 2006;18:741-5.

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AQ1:Kindly check author name does not match with reference list

AQ2:Kindly review the sentence.