INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

Acute toxicity of NPK fertilizer on soil ecosystem using earthworm, *Drawida willsi* as a test specimen

Anindita Bhattacharya[1,2] and Sanjat Kumar Sahu[2]

[1] Department of Earth Science (Environmental Science),

University of Science and Technology, Meghalaya, India - 793101.

[2] P.G. Department of Environmental Sciences, Sambalpur University, Odisha, India - 768018.

ABSTRACT

Use of agrochemical is now considered to be a serious concern in many regions of the world. Among agrochemical, fertilizer production had been increased at an annual rate of 3.1% throughout the world in last decade. This must have an adverse effect on the soil ecosystem. Unfortunately, very few studies were conducted on the impact of fertilizer on the soil health. No study was conducted for the acute toxicity of NPK, which have been widely used in the agricultural field of India on earthworm *Drawida willsi*. Hence the present study was undertaken to study the acute toxicity of NPK on soil ecosystem using earthworm *D.willsi* Michaelsen as a test animal which constitutes the dominant earthworm in crop fields of India. For this, soil and earthworm were collected from such agricultural field where there had no record of input of agrochemicals. Different concentrations of NPK were prepared in dilution of water which was then added to the experimental sets. Then ten number of earthworms of each age-sex group (juvenile, immature, adult) were gut evacuated and added to five replicates (polythene packets) for each concentration of NPK fertilizer. For the control set, only water was given and ten numbers of earthworms were added. The experiment was conducted at 20±2g% soil moisture and 25±2°C soil temperature. The Finney's Probit method was followed to evaluate 96h LC_{50} values. Study found that the juvenile, immature and adult worms of *D. willsi* survived in soil containing up to 250, 370 and 490 mg/kg of NPK respectively. But 100% mortality of juveniles were recorded when worms were exposed to concentration of 490 mg/kg of NPK. However at a concentration of 640 mg/kg and 730 mg/kg, 96% of immature and 92% of adult mortality was observed respectively. The 96 h LC_{50} values with their 95% confidence limits for juvenile, immature and adult worms were 374.28 (374.27-374.29), 512.86 (512.85-512.87) and 616.60 (616.59-616.61) mg/kg respectively. This clearly indicates that the recommended dose of NPK was safe since there was no mortality of earthworm. However, this finding is not enough to draw a concrete conclusion about the toxicity of NPK since there must be some sub-lethal effect which may cause an adverse effect on the growth, reproduction and metabolism of earthworm. So, proper care should be taken on doses during use of NPK in the agricultural field.

Key words : Agrochemical, NPK, acute toxicity and *Drawidawillsi.*

INTRODUCTION

India is the third largest fertilizer consumer in the world, after China and the United States. India is so far the main consumer of fertilizers in the South Asia region. The intensity of fertilizer use in India increased from 95 to 128 kg/ha during 2002–2006.[12] During 2002–2007, India consumed 116 million MT of nutrients, which represented 74% of regional consumption (South Asia) and 12% of global consumption. By macronutrient, India is the main consumer of all three macronutrients, accounting for 72% of the total amount of nitrogen, 76% of phosphate and 84% of potash consumed South Asia. The total NPK consumption during 2006-07 was 21 million tonnes in India.[16]

The agrochemicals, pesticides and inorganic fertilizers are used in a huge rate in the agricultural field to augment the crop productivity. Soil is a habitat supporting different life forms. It is also a

reservoir of all the agrochemicals. When these agrochemicals enter the soil, they may disturb the soil ecosystems by impairing the physical, chemical and biological components specially the non-target beneficial microorganisms and earthworms.[3,9]

Earthworms constitute more than 80% of the invertebrate biomass in most of the agro-ecosystems of the world and plays important role in improving the structure and fertility of the soil through their feeding, casting and burrowing activities.[8,13] Due to their beneficial role in agroecosystem, earthworms are used as indicator species for monitoring the impact of pollutants, changes in soil structure and agricultural practices.[10,15]

A number of studies were conducted on the acute toxicity of NPK on the aquatic ecosystem. Most of these studies were conducted on fish.[4-7,14,18,20] Very few studies were conducted on the toxicity of NPK using *earthworms Eisenia foetida* as test animal by Paper contact method.[1,2] But no study was conducted to test the toxicity of NPK on the soil ecosystem till date using tropical earthworm *Drawida willsi*, Michaelsen as a test animal which constitutes the dominant earthworm in crop fields of India. Therefore, earthworm *Drawida willsi* was undertaken in the present experiment as the test animal to find out the toxicity level of NPK on soil ecosystem.

MATERIALS AND METHODS

Soil and Earthworm: For the experiment, earthworm (*D.willsi*) and soil were collected from an upland non-irrigated paddy field which had no record of input of agrochemicals.

The soil was of laterite type with sandy loam texture. It had a pH of 6.8, organic matter (g%) 4.7, nitrogen g (%) 0.22 and a C/N ratio of 12.27. Prior to use, the soil was air dried and sieved. Then several experiment sets were prepared each with 500 gm soil in the polythene packet.

After collection, the earthworms were cultured for one month at their native soil in the laboratory condition at moisture (20±2g%) and temperature (25±2ºC) with a diet of 10% organic matter (cow dung + leaf litter).[19] Then earthworms were removed from culture pots and gut cleaned by immersing them in glass petriplates having 30 ml of tap water in 25±2°c for 24h. Then they were categorized into three age classes (juveniles: <2cm, immature: ≥2<4cm and adults : ≥4cm) on the basis of size and presence or absence of genital papilla and clitellium.

Fertilizer: The recommended agricultural dose (mg/kg) calculated for urea, superphosphate, potash came to be 79.04, 39.52 and 39.52 respectively and for NPK it came to be 160 (NPK in the ratio of 2:1:1).

Mixtures containing all the three principal nutrients (N, P and K) are termed complete fertilizers. According to EEC Guidelines, NPK fertilizers must contain at least 3% N + 5% P_2O_5 +5% K_2O and at least 20% total nutrients. The most commonly used grades are:

Nutrient Ratio 1:1:1
Nutrient Ratio 1:2:3 and 1:1.5:2
Nutrient Ratio 1:1:1.5-1.7
Nutrient Ratio 3:1:1 and 2:1:1

Preparation of NPK was made as follows **:** (i) 80 mg/kg N+40 mg/kg P+40 mg/kg K=160 mg/kg NPK, (ii)90 mg/kg N+50 mg/kg P+50 mg/kg K =190mg/kg NPK and so on. These were added to the soil surface and then mixed thoroughly with enough water to ensure a homogeneous mixture. The same procedure with only water was applied to prepare the control set.

Toxicity Test: Different concentrations of NPK in a ratio of 2:1:1 were prepared in dilution of water. These were added to the experiment sets and then mixed thoroughly to ensure a homogeneous mixture with soil. Then ten healthy gut evacuated earthworms of each age group (juvenile, immature, adult) were added to five replicates (polythene packets) for each concentration of NPK fertilizer. The experiment was maintained at 20±2g% soil moisture and 25±2°C soil temperature. Earthworm deaths were recorded and Finney's probit method was followed to calculate 96h LC_{50} values.

RESULT AND DISCUSSION

The experiment found a wide variation of toxicity on juvenile, immature and adult earthworms due to NPK. Details of the toxicity with respect to different dose and replicates are given below.

Effect on juvenile earthworm

No mortality was reported when juvenile *D. willsi* earthworms were exposed to 250 mg of NPK fertilizers per kg of soil. But about 5% mortality was recorded at the exposure of 280 mg/ kg of NPK in soil. Gradually the mortality increased with the increase of doses and 100% mortality was observed at the dose of 490 mg/kg of NPK to soil.

There was a wide variation in toxicity of NPK on juvenile earthworm with respect to dose conducted in five replicates (Table-1). About 5% mortality of earthworms were recorded when they were exposed to 280 mg/kg of NPK in all the five replicates. The mortality increased to 19% when they were exposed to 310 mg/ kg of NPK to soil followed by 30% at 340 mg/kg, 42% at 370 mg/kg, 58% at 400 mg/kg, 76% at

430 mg/kg, 92% at 460 mg/kg and all the juvenile earthworms died at 490 mg/kg of NPK to soil.

The Finney's Probit analysis indicates the 96h LC_{50} values with 95% confidence limit for juvenile earthworm was of 374.28 with a range of 374.27 to 374.29 mg/kg of soil (Fig-1, Table-1).

Effect on immature earthworm

There was no mortality of immature earthworm when exposed to NPK at the dose up to 370 mg/kg to the soil. But mortality started when they were exposed at 400 mg/kg soil and 100% mortality was observed at the dose of 670 mg/ kg soil.

Table 1

96 h toxicity test for *D. willsi* exposed to different concentrations of NPK

Age-class	Concentration (mg a.i./kg dry soil)	Mortality	Emperical probit	Expected probit	96 h LC_{50} with 95% confidence limit
JUVENILE	250	0	--	--	374.28 (374.27-374.29)
	280	5	3.36	3.2	
	310	19	4.12	3.8	
	340	30	4.48	4.48	
	370	42	4.8	5.25	
	400	58	5.2	5.6	
	430	76	5.71	5.95	
	460	92	6.41	6.62	
	490	100	8.09	7	
IMMATURE	370	0	--	--	512.86 (512.85-512.87)
	400	2	2.95	3	
	430	10	3.72	3.72	
	460	22	4.23	4.23	
	490	44	4.85	4.6	
	520	56	5.15	5.1	
	550	68	5.47	5.47	
	580	78	5.77	5.8	
	610	84	5.99	6.25	
	640	96	6.75	6.6	
	670	100	8.09		
ADULT	490	0	--	--	616.6 (616.59-616.61)
	520	8	3.59	3.7	
	550	24	4.29	4.15	
	580	36	4.64	4.55	
	610	48	4.95	4.95	
	640	56	5.15	5.3	
	670	72	5.58	5.65	
	700	80	5.84	5.95	
	730	92	6.41	6.3	
	760	100	8.09		

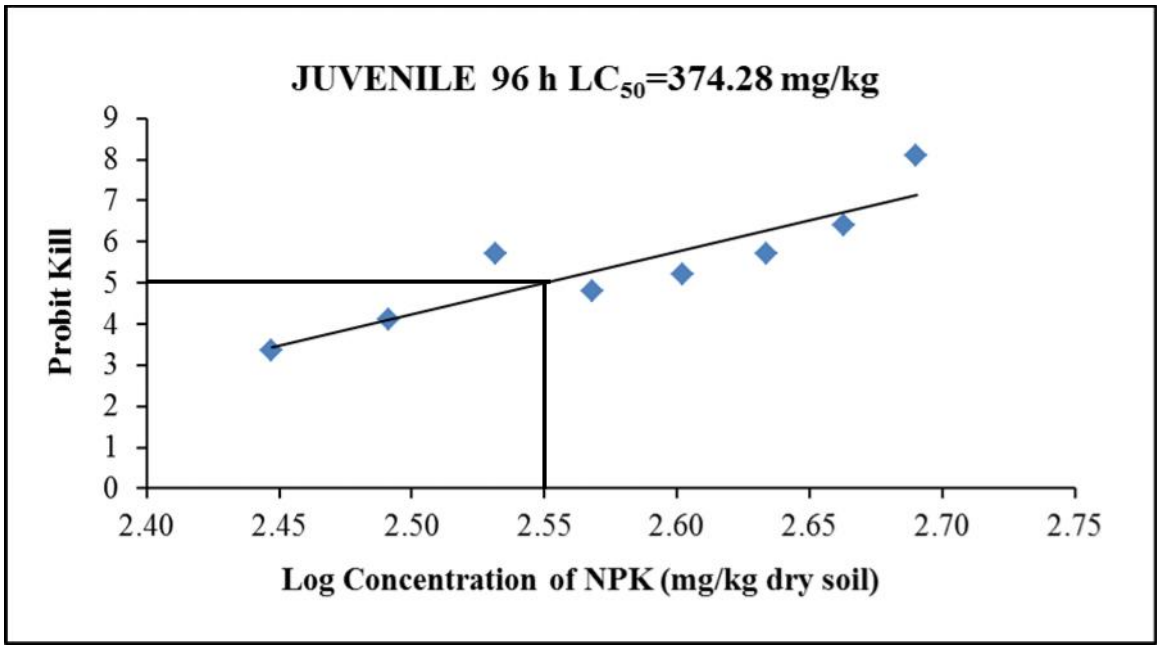

Fig 1
96 h toxicity test for juvenile *D.willsi* exposed to different concentrations of NPK

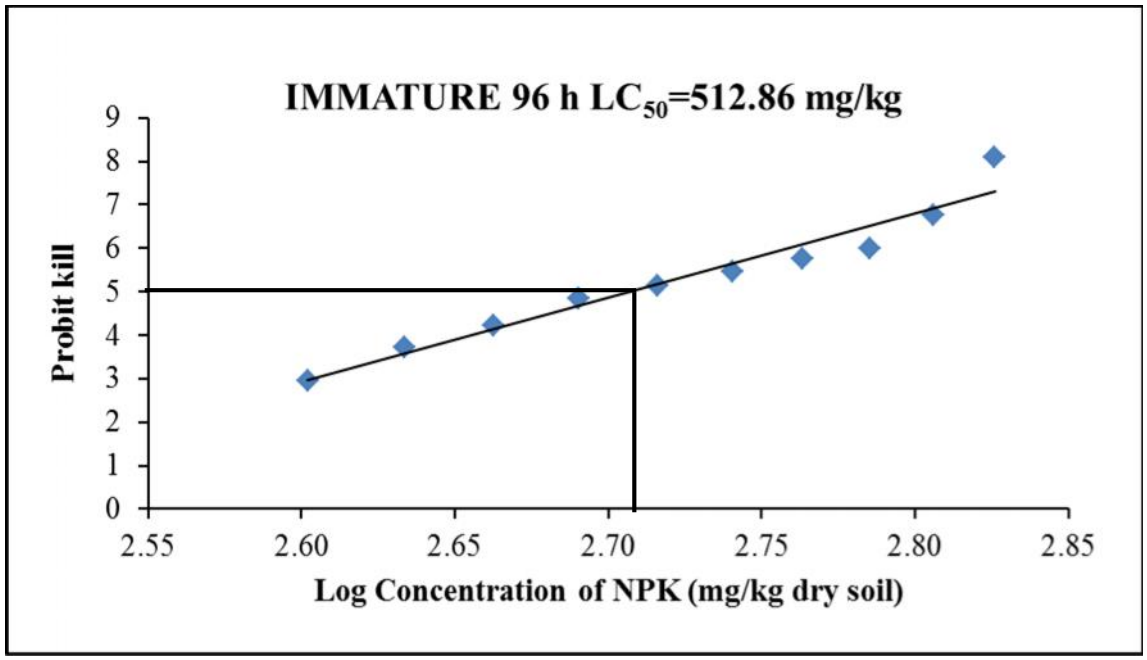

Fig 2
96 h toxicity test for immature *D.willsi* exposed to different concentrations of NPK

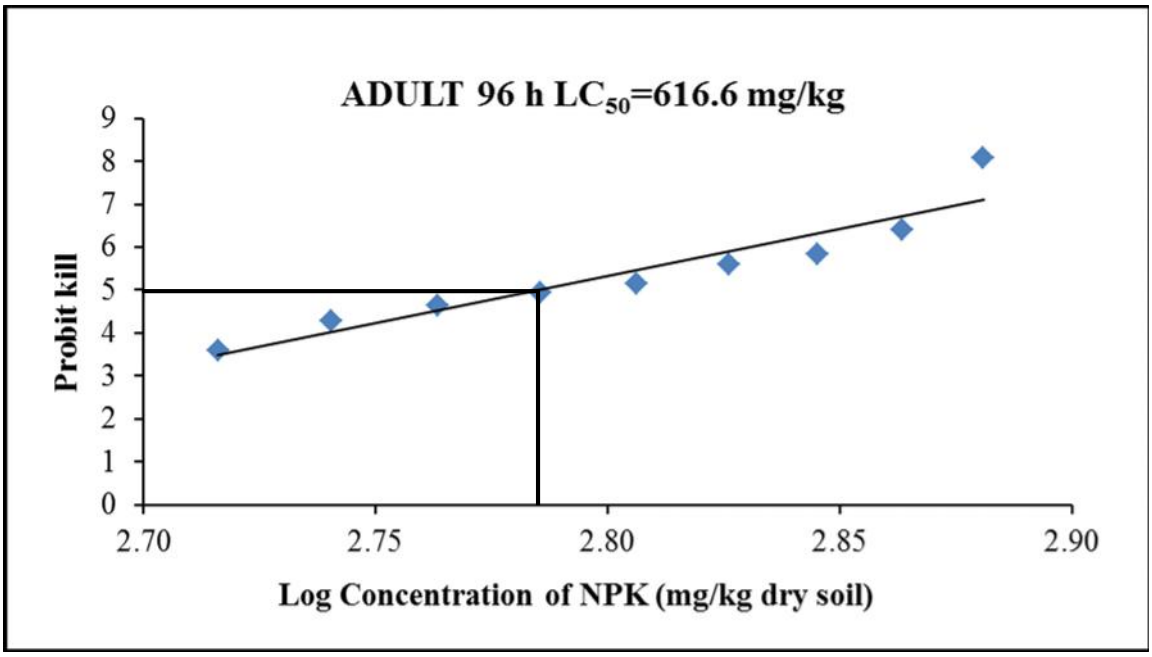

Fig 3
96 h toxicity test for adult *D.willsi* exposed to different concentrations of NPK

Effect on adult earthworm

It was observed that all the adult earthworms survived when exposed to NPK up to the dose of 490 mg/kg to soil. But mortality started at the exposure of 520 mg/ kg to soil. The mortality gradually increased with the increase of doses and 100% mortality was observed at the dose of 800 mg/kg of NPK to soil.

A distinct variation of adult earthworm mortality was also recorded with respect to different doses of NPK conducted in five replicates (Table-1). About 8% mortality was recorded when they were exposed at 520 mg/kg followed by 24% at 550 mg/kg, 36% at 580 mg/kg, 48% at 610 mg/kg, 56% at 640 mg/kg, 72% at 670 mg/kg, 80% at 700 mg/kg, 92% at 730 mg/kg, and 100% at 760mg/kg soil.

The Finney's Probit analysis indicates the 96h LC_{50} values with 95% confidence limit for adult earthworm was of 616.6 with a range of 616.59 to 616.61 (Fig-3, Table-1).

CONCLUSION

The present investigation found that the variation in doses of NPK had upshot a discrepancy in the degree of mortality in different age class of *D. willsi*. There was 100% mortality at 490 mg NPK/ kg for juvenile, 670 mg NPK/kg of soil for immature and 760 mg NPK/kg of soil for adult. The 96h LC_{50} value was found to be 374.28 for juvenile, 512.86 for immature and 616.6 for adult *D. willsi*. This clearly indicates that the recommended dose for NPK (160 mg/kg) is safe so far point of mortality is concern.

Although there was no report of mortality of earthworm at the recommended dose, the use of NPK fertilizer cannot be safe. This is because, the NPK contains phosphates which undergoes bioaccumulation and may cause severe toxicity. So it must have some sub-lethal effects even at the recommended agricultural doses.[17]Therefore, further test on sub-lethal effect of NPK on parameters like growth, reproduction, metabolism etc. are required to draw a safe conclusion. However, it is interesting that the ccomposite fertilizer (NPK) is still classified as the non-hazardous material.[11]

Beside this, the phosphate contains of NPK also accumulates in the water bodies through surface run off resulting eutrophication. This may cause a complete destruction of aquatic ecosystem due to complete death of the biological system.

In conclusion, the present experiment suggests that although the NPK is not very toxic to earthworm at the recommended doses still it could have some sub-lethal effect that would affect the agro-ecosystem.

ACKNOWLEDGEMENT

The authors are thankful to the DST, Govt. of India, New Delhi for providing infrastructural facilities through FIST Grant. Anindita Bhattacharya is thankful to Sambalpur University for a fellowship.

REFERENCES

1. Abbiramy KSK and Ross PR. Determination of acute toxicity of NPK fertilizers to Eisenia foetida using a simple paper contact method. Int. J. Sci. Res, 2013a; 2(2), 415-417.
2. Abbiramy KSK and Ross PR. Validation of tropical artificial soil ecotoxicological studies on Eisenia foetida against combine fertilizers. Int. J. Innovative Res. And Studies, 2013b; 2(7): 212-227.
3. Anderson JR. Pesticide effects on non-target soil microorganisms. In: Pesticides Microbiology, I.R. Hill and S.J.L. Wright (Eds.) Academic Press, London, 1978: 313-33.
4. Asuquo IE and Essienibok MA. Acute Toxicity of NPK 15:15:15 Fertilizer on the Fingerlings of Heterobranchus bidorsalis. West African Journal of Applied Ecology, 2014; 22(2): 31–43.
5. Ayuba VO, Okomoda VT and Ogah SIO. Acute toxicity of NPK 20:10:10 to fingerlings of Clarias gariepinus. Banat's Journal of Biotechnology, 2014; 5(9): 34-40.
6. Capkin E, Kayis S, Boran H and ltinok I. Acute Toxicity of Some Agriculture Fertilizers to Rainbow Trout. Turkish Journal of Fisheries and Aquatic Sciences, 2010; 10: 19-25.
7. Chukwu LO and Okpe HA. Differential response of Tilapia guineensis fingerlings to inorganic fertilizer under various salinity regimes. Journal of Environmental Biology, 2006; 27(4): 687-690.
8. Dash MC. Role of earthworms in decomposer system. In: Glimpses of Ecology. J. S. Singh and B. Gopal (Eds). International Scientific Publishers, New Delhi, 1978: 309-406.
9. Edwards CA and Bohlen PJ. The effects of toxic chemicals on earthworms. Rev. Environ. Contam. Toxicol., 1992; 125:23-99.
10. Eijsackers H. Earthworms in environmental research. In: Earthworms Ecology, C.A. Edwards (Ed.), CRC Press, Boca Raton, FL, 2004.
11. EPC (European Parliament and of the Council). Directive 1999/45/EC of the European Parliament and of the Council of 31 May 1999 concerning the approximation of laws, regulations and administrative provisions of the member states relating to the classification, packaging and labelling of dangerous preparations. OJEC L200 of 1999-07-20, 1999, 1–68.
12. Harnandez MA and Torero M. Fertilizer Market Situation: Market structure, Consumption, and Trade Pattern and Pricing behaviour. FPRI (International Food Policy Research Institute), 2011, 1-76.
13. Lee KE. Earthworms, their ecology and relationship with soils and land use. Acad. Press, Australia, 1985.
14. Maurya GK, Malick C, Mondal R and Gambhir L. Study of acute toxicity of inorganic fertilizer NPK (15:15:15) on cat fish, Heteropneustes fossilis. International Journal of Applied Biology and Pharmaceutical Technology, 2016; 7(2): 243-253.
15. Paoletti MG. The role of earthworms for assessment of sustainability and as bioindicators. Agric. Ecosyst. Environ., 1999; 74(1-3): 137-156.
16. Rao AS and Reddy KS. Soil: Deterioration in physical, chemical and biological qualities. In: The Hindu Survey of Indian Agriculture, Chennai, India, 2008, 105-109.
17. Reddy MV and Goud AN. Impact of inorganic fertilizers on the earthworm population density of wetland rice (Oryza sativa) agroecosystems. Proc. Nat. Symp. Ecotoxico. 1987; 154-158.
18. Sangeetha S, Sujatha K, Senthilkumaar P, Kalyanaraman V and Eswari S. Acute toxicity of some agriculture fertilizers to fingerlings of Catla catla. Indian J. Sci. Technol., 2011; 4(7): 770-772.
19. Senapati BK and Dash MC. Functional role of earthworms in decomposer subsystem. Trop. Ecol., 1984; 25(2): 54-73.
20. Uhuo CA, Nwele DE, Odikamnoro OO, Ibiam GA and Azi SO. The Toxicity Effect of N.P.K. Agro-Fertilizer on Oreochromis niloticus Fingerlings, the Pysiological Impact. Journal of Biology, Agriculture and Healthcare, 2013; 3(9): 5-8.