International Journal of Agriculture, Environment and Bioresearch

Vol. 10, No. 02; 2025

ISSN: 2456-8643

THE USE OF SIGMOIDAL DOSE RESPONSE IN ASSESSING ECOTOXICOLOGICAL RISK OF AGROCHEMICALS ON MICROBIAL ENZYME ACTIVITY IN SOILS

Ferisman. Tindaon[1*], Ferlando Jubelito Simanungkalit[1], Dunan Naibaho[1], Yanto Raya Tampubolon[1], Ferlist Rio Siahaan[1], Shanti Desima Simbolon[1] and Parlindungan Lumbanraja[1]

[1]Agroecotechnology Department, Faculty of Agriculture, University of HKBP Nommensen, Medan, Indonesia Jl. Sutomo No. 4A Medan 20234 Indonesia.

*Corresponding Author: E-mail: Ferisman.Tindaon@uhn.ac.id

https://doi.org/10.35410/IJAEB.2025.5968

ABSTRACT

Ecotoxicological risks of non-target soil microbial processes, caused by three nitrification inhibitors (Nis) 3,4dimethylpyrazolephosphate=DMPP, 4-Chlor-methylpyrazole= ClMP and dicyandiamide-DCD) were estimated in three different types of soils by employing the dehydrogenase activity (DHA). DHA was spectrophotometrically quantified. NIS concentration dependent and for evaluating inhibition effects no observable effect level=NOEL, as well as effective dose ED10 and ED 50(10% and 50% inhibition) were calculated and presented in dose response curves. The inhibition is most distinct in sandy soils. At an about 30–70 times higher NI application rate than the recommended field application rate must accumulate in soils before the NOEL for microbial non target processes is surpassing and harmed microbial cells become observable. ClMP exhibited the strongest influence on non-target microbial soil processes. It is suggested that the data presented here could be very useful in helping to set permissible limit for agrochemicals soil pollution. An Ecological Dose Range to describe the increased rate of inhibition upon increasing concentrations of a pollutant was proposed. Remarks were made about the way this model must be used together with applications in other fields of soil biological research.

Keywords: Dose Response, Ecotoxicology, Agrochemicals, Microbial Activity

1. INTRODUCTION

Recently, dose response curves are widely used to assess the ecotoxicological effect of agrochemicals on microbial activities in agricultural research and application. A dose-response design can provide evidence of causal effects between treatment and responses. In laboratory studies, agrochemicals (nitrification inhibitors) were added to soil samples, and the effect on soil microbial activities was measured after a period of time (Rahmatpour et al., 2017:Shin et al., 2012; Tindaon et al.,2011, 2012; 2013;2019). There are several common techniques for measuring the activity, number or biomass of microbes in soil. The most basic is the direct count, where microbes are extracted from soil in a liquid, sometimes dyed to illustrate particular groups, and then counted on a slide under the microscope. Another method for determining microbial numbers is the plate count. Again, an extract is made from soil and a small amount is placed in a Petri dish and incubated. Various growth media can be used in the dish to select for or against certain organisms. The growth of the microbes into visual colonies is then measured. Plate counts tend to underestimate microbes because only 1- 10% of soil organism types can grow on artificial medium, and thus many go undetected. Some methods of measuring soil microbial activities were used to assess their suitability for testing the side effects of agrochemicals on microbial activities (Rahmatpour et al., 2017, Tindaon et al., 2012;2013;2019). Reproducibility

Vol. 10, No. 02; 2025

ISSN: 2456-8643

and the sensitivity of the methods (and activities) were very important. In these investigations, the agrochemicals like nitrification inhibitors (DMPP, ClMPP and DCD were applied at different dosages in different types of soils. Addition of nitrification inhibitors (NIs) to fertilizers have beneficial effect on reducing nitrate leaching and nitrous oxide emission and as a result increase plant growth (increase N use efficiency. The use of nitrifications inhibitors expected will be able to control the microbial ammonium oxidation which convert ammonium to nitrate, decrease N leaching, improve efficiency of N use by crops and decrease the nitrous oxides emission. Thereby N use ecologically will be more efficient. Further, nitrification inhibitors use in agriculture should be recommended in low concentration and capable to control nitrate supply to crop so that avoid the excess of nitrate supply in soils. The inhibitor have the specific influence that is only inhibit the nitritation (oxidize the ammonia become the nitrite) and not for nitratation (oxidize the nitrite become the nitrate) so that accumulation can be avoid. The Inhibitor should be bacteriostatic and not a bakteriocide which killing certain microorganism in soils like Nitrosobacter spp, Nitrosococcus sp. Furthermore, agrochemicals such as NI can alter microbial diversity or function, which may indirectly affect soil fertility and nutrient balances. Finally, NI have no negative influence on common microbial activity which is nontarget in soils. Various standard methods have been recognized to know the side effects of agrochemicals use to environment which can be measured either in laboratory and also the field (Ahmad et al., 2024). The overall soil microbiological activity describing enzyme is the dehydrogenase activity (DHA) which acts as intercellular enzyme and transfers metabolically H^+ and $e^-$and was employed inter alia to describe the microbiological activity in forest soils (Quilchano and Maranon 2002). Total bacteria and archaea numbers, counted dyed under the microscope or after cell growth on selective media on plates counts tend to underestimate microbes as molecular biological techniques exhibit (e.g. Kisand andWikner, 2003). It is assumed that until now only 1-10% of the present soil bacteria and archaea can be grown on artificial media and thus more appropriate in evaluatingNI side effects at actual soil situations seem to be enzymatic methods as DHA, DRA and N cycle concerned NA and PDC (Allison et al., 2008; Araujo et al., 2009;Baldrian, 2009; Ferreira et al., 2013; Santric et al., 2014; Zannatta, et al, 2007). These standard methods have been recognized to know the side effects of chemicals use to environment which can be checked either in laboratory and also in the field (Malkomes, 1997a,b, Tindaon et al., 2012; 2019).

The effects of agrochemical as soil pollutants have been investigated frequently by measuring the decrease in the rate of soil microbiological activity upon increasing the concentration of nitrification inhibitors. In laboratory studies, NIs were added to soil samples, and the effect on soil microbial activities was measured after a period of time. The number of concentrations used varied in rate of concentrations used. The dehydrogenase in the presence of added NIs was mainly expressed as a percentage of the dehydrogenase of the unamended soil samples.

When soil microbial activity (DHA) rates or soil enzymatic activity was measured in the vicinity of local agrochemicals sources a negative correlation was found between the concentration of the NIs and the microbial activity measured, In our experiments we have more often found sigmoid relations on a logarithmic scale rather than linear relations. Therefore, in this note, a logistic response curve is proposed.

Vol. 10, No. 02; 2025

ISSN: 2456-8643

2. MATERIALS AND METHODS

Model experiments

Soil samples from 3 differently textured soils (a loamy clay, a loam and a loamy sand) were sampled (0-20 cm), air dried, sieved (< 2 mm) and carefully homogenized. The clayey and loamy soil were taken from the Experimental Station of the Department of Agronomy and Plant Protection, Justus Liebig University, Giessen, Germany, whereas the loamy sand samples were obtained from the BASF Agricultural Center, "Limburgerhof". The used soils in the incubation experiments were analyzed physico-chemically by standard methods and their properties are shown in Table 1.

Table 1. Physico-chemical properties of the differently textured soils used in the model experiments

Parameters	Type of Soil		
	silty clay	silt	Loamy sand
C_{total} (%)	1,35	1,30	0,70
C_{H2O} (%)	0,40	0,55	0,27
N_{total} (%)	0,15	0,15	0,08
C/N	10	9	9
pH $_{H2O}$	6,30	7,00	7,00
pH $_{KCl}$	6,00	5,50	6,40
Fraction (%)			
Clay	51	24	6
Loam	41	46	19
Sand	8	30	75

Nitrification inhibitors

DMPP (purity 99.9 %) and ClMP (99.7 %) were obtained from the BASF SE, Ludwigshafen, Germany, while DCD (purity 96%) was purchased from SKW Trostberg AG, Trostberg Germany. The experimentally by the marketing companies found field recommendation rates of 0.36, 0.25 and 10 µg g^{-1} dry soil for DMPP, ClMP and DCD, respectively, calculated for 90 kg N ha^{-1}, are formulated on ammonium sulphate before application. Besides the field recommended NI concentrations we tested in the model experiments the concentration 0 (control) and 5, 10, 25, 50, 100, 250, 500, 750 and 1000 times higher concentrations than the recommended field rate.

DMPP effect on a nitrifying consortium

A nitrifying consortium enriched from the top soil of the Experimental Station, Department of Agronomy and Plant Protection, Justus Liebig University, Giessen, Germany clayey was in liquid culture confronted with the field recommended DMPP and a 10 times higher concentration and during an incubation period of 75 days regularly pH NH_4^+, NO_2^- and NO_3^- measured. At the end of the experiment, picture of the participating bacteria by transmission electron microscopy were made for documenting morphological changes. For methodological details and results, see Benckiser et al. (2013).

Vol. 10, No. 02; 2025

ISSN: 2456-8643

Soil microbial parameter.

The dehydrogenase activity (DHA) was assayed according to the INT method (ISO 23753-2:2005) in air dried soil samples (2.5 g), weighed into test tubes (50 ml; 5 replicates), supplemented with 2.5 ml of the alternative electron acceptor 2-(4-iodophenyl-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT)-Tris buffer solution and carefully mixed with the NI examined (whirlmix). The tubes were sealed (rubber stoppers) and incubated in semi-darkness (4 h, 25 ^{0}C), because INT is sensitive to light. Control tubes without NI were treated identically. Intracellular dehydrogenase activity reduced INT to insoluble iodonitrotetrazolium formazan (INF), which was subsequently extracted with 10 ml tetrahydrofurane (Merck, Darmstadt) by shaking the tubes overhead (1 h). The extracts were kept for 2 h in a semi-dark room, homogenized, filtered (Schleicher & Schuell, Dassel) and the extinction of INF spectrophotometrically measured against the blank (ZEISS PM2-DL; 436 nm). DHA was expressed in µg INF g^{-1} dry soil h^{-1} by using an INF calibration curve.

Logistic Response Models and Dose Response Relationship

Non-linear regressions, analysis of variance (ANOVA) and Fisher's least significant difference pair-wise comparison tests were applied for describing dose response-relationships (Stephenson et al. 2000). ED_{10} and ED_{50} values denote the doses of the NI concerned causing 10% and 50% inhibition, of nitritation, respectively. The highest dose at which no effect can be observed (NOEL= No Observed Effect Level) describes the critical concentration that does not cause adverse effects. Significant differences at $P < 0.05$ level were obtained by using SigmaPlot and SigmaStat Software (SPSS Inc). NOEL, ED_{10} and ED_{50} were calculated by equation (1)

$$Y = a/ \{1 + \exp [- (X_t - X_0)/b]\} \quad (1)$$

Y equals the maximum response (a) divided by 1 + exp [- $(X_t - X_0)/b$]. Xo and Xt are the log NIs doses at the beginning and end of the experiment and b is a constant describing the NI-influence (Richter et al. 1996). The toxicity index (TI), calculated by dividing NI-concentrations causing ED_{50} through the concentrations causing ED_{10} effects, describes the intensity in the decrease of the parameter concerned (Liao et al 2005).

3. RESULT AND DISCUSSION

Dose response relationships

Mathematical model for ecotoxicological test on the NOEL, ED10 and ED50 for the three NIs data can be studied constructively by using Sigma plot and Sigma stat. So that approach method showed the threshold of ecotoxocological parameters the substances were determinable (Tables. 2). If using ordinary linear equation regression, hence determination assess the NOEL from measurement data was not at all enabled. For example, a semilogarithmic dose response relationship between three NIs (DMPP, ClMPP, DCD) and dehydrogenase activity in clay soil is presented in Figure 1. By using the equation for dose response curve (Moreno et. al.., 2001) in Sigma Plot Program where as $Y= a/(1+ \exp(-(Xt-Xa)/b))$, it is possible to calculate critical Value for NOEL, ED_{10} and ED_{50}. Y= response, a = maximal response, Xo and Xt = log dose of used NIs according to time.

Vol. 10, No. 02; 2025

ISSN: 2456-8643

Table. 2 Dehydrogenase activity in soil samples from controls, that is soil not treated with the used nitrification inhibitors

Soil type	Dehydrogenase activity[1)] (µg INF g^{-1} dry soil and h^{-1})
Silty clay	431.6 ± 3.4
Silt	274.2 ± 2.3
Loamy sand	121.0 ± 0.9

[1)] = Average of 5 replicates

The DHA estimates in the control soils, which have seen NI never before (Tab. 2) are in agreement with data published by others with similar soils (Makoi and Ndakidemi 2008). Thus, for evaluating NI side effects on non-target microbial activities in the used clayey, loam and sandy soil are suited to give reliable information about DMPP, ClMP and DCD effects on the DHA describing the overall soil metabolism after application in increasing concentrations. Fig. 1 presents semi-logarithmically in % of the control the side effects of DMPP, ClMP and DCD on the DHA in the clayey (A), loam (B) and sandy soil (C) and it can be concluded that (1) the benchmark between affecting and non-affecting, the NOEL values, are surpassed by DMPP and ClMP at lower concentrations than at DCD, (2) NOEL-values for all 3 NIs could be significantly ranked clayey soil > loam soil > sandy soil whereby in the clayey soil DMPP, ClMP and DCD inhibit the DHA first from approximately 50-times (DMPP, ClMP) or 250-times higher concentrations (DCD) on than those generally applied in the field (ca. 0.36 µg DMPP, 0.25 µg ClMP, 10 µg DCD g^{-1} dry soil). In culture solution the target organisms, the nitrifiers, are severely and seemingly irreversibly damaged by a 10 times higher DMPP concentration than the field recommended one (Benckiser et al., 2013) and if this observation is compared to that made witht increasing DMPP, ClMP and DCD concentrations added to the clayey, loam and sandy soil, then it becomes obvious that soils smooth assumingly due to NI absorption, diffusion and degradation phenomena NI effects (Azam et al., 2001; Weisske et al., 2001). In the loam soil DMPP, ClMP and DCD, inhibit DHA from a a 25-times higher concentration on than the basic application rates. The NOEL values in this soil starting to be surpassed from 9 µg g^{-1} (DMPP), 6 µg g^{-1} (ClMP) and 250 µg g^{-1} (DCD) on compared to 3.6 µg DMPP, 2.5 µg ClMP and 100 µg DCD g^{-1} dry in the sandy soil. Apparently, the sandy and loam soils show earlier NI side effects on DHA than the clayey soil. ClMPP, not marketed because of its chlorine component, expressed the strongest side effect potential among the 3 tested NI and a longer running NI inactivation can be assumed.

Vol. 10, No. 02; 2025

ISSN: 2456-8643

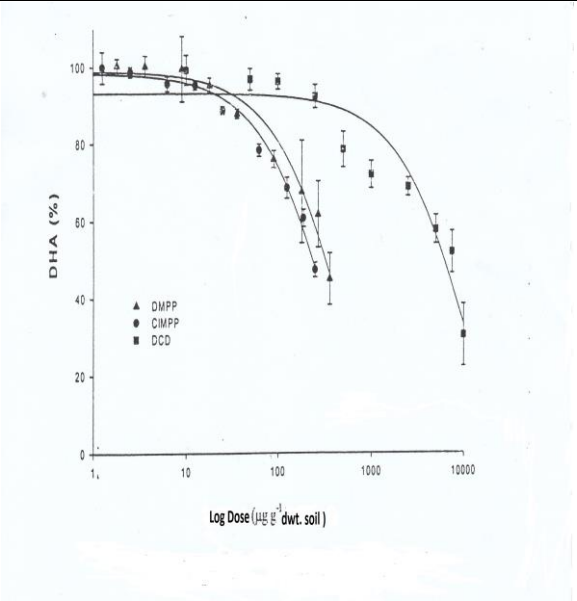

Fig.1. The effect of increasing the concentration of NI DMPP, ClMPP and DCD on dehyrogenase activity (% control) in clayey soil (semilogaritmic, the dose in the log). Dosage recommendations, were 0.36 µg DMPP; 0.25 µg ClMPP and 10µg DCD per gram dry soil.

Table 2 compares the DMPP, ClMP and DCD related and DHA concerning NOEL-, ED_{10}- and ED_{50}-values and it is obvious that the clayey soil with its highest absorption potential shows NOEL-, ED_{10}- and ED_{50} effects-values first when the applied NI concentrations were higher than those added to the loam and sandy soil. The soil texture protects non-target soil organisms from being NI side affected by NI. Despite under the given experimental conditions the recommended DMPP, ClMPP and DCD application rates are far below those exhibiting side effects and thus possible hazards on DHA, is not likely even in the sandy soil, it should be kept in mind that we are just in the beginning to understand the behaviour and activities of soil bacteria, archaea, protozoa, fungi and their syntrophy in biofilms and soil microbial communities (Benckiser and Bamforth 2010; Bannert et al. 2011). Nevertheless, though DMPP that indiscriminately binds to the complex of membrane-bound proteins inclusively the AMO and DCD that blocks the electron transport in the cytochromes of AMO during the conversion of NH_3 to hydroxylamine lower nitrate availabilities and N_2O emissions after field application by 26-49%, the European Commission (EC) hesitates in a cost/benefit analysis of farming practices where NI stabilized N-fertilizers were evaluated to recommend the application of those, because they are still relatively expensive, their N-saving effectiveness insufficiently tested and cereal and maize crop yield improvements not conclusively documented (PICCMAT, 2011).

The reported findings that the tested NI by us not very likely harm at the field recommended concentration important soil processes is supported by similar findings of others (Mahmood, et al, 2005). On 16S RNA basis studied bacterial community structures before and after DCD application indicate that the nitrification inhibitor dicyandiamide did not change the soil bacterial phyla essentially (O'Callaghan, et al, 2010) and an Uzbekistanian study with potassium oxalate as nitrification inhibitor found an increase in oligonitrophilic bacteria and cellulose degradation activity, while nitrifying and denitrifying bacteria decreased in numbers

Vol. 10, No. 02; 2025

ISSN: 2456-8643

(Egamberdiyeva *et al*., 2001). On the other hand, Austin *et al*. (2006) showed that after nitrapyrin application to an undisturbed semi-arid steppe organic matter decomposition was lowered and the authors stated that the N species ratio (i.e. ammonium vs. nitrate) may be of more importance concerning carbon cycling and ecosystem functioning than the quantity of N present in the system. Thus, a reduced nitrification is impacting the soil carbon cycle and such interactivities between DMPP application and carbon cycle also Weiske et al. (2001) found in their field studies. Such still little understood NI effects on the carbon cycle indicate that there are further needs to study more detailed side effects of nitrification inhibitors on nitrogen dependent nutrient turnovers in soil ecosystems.

More recent studies demonstrated that the efficacy of DMPP was closely related to soil clay and organic constituents, which evidently play a major role in NI inhibition (Austin, et. al, 2006: Barth, et. al, 2006 and 2008). Once the nitrification inhibitor is in the soil, it may be gradually broken down by soil microbes what means a slowed but wished disappearance, a timely limited nitrification inhibition (Sahrawat, 2004). NI performance during plant free and early growth periods, during periods when plants are not able to compete for nitrate may vary from agroecosystem to agroecosystem whereby a major role plays the soil temperature, organic carbon availability, the applied NI concentration, and the superiority of granulated DMPP-fertilizer application over liquid DMPP-application (Di and Cameron 2004; Ali et al., 2008; Barth, et. al., 2006 and 2008; Li, et. al., 2008; Mahmood et al., 2011).

The results of Tab.3 reveal that between the 3 tested NI, DMPP, ClMPP and DCD no significant differences in respect to the calculated NOEL, ED10 and ED50-values exhibited, though the ClMPP side effect potential on nontarget activities is tendatively a little stronger than that of DMPP and DCD, perhaps due to the chlorine component(Mc Carty 1999). Even a NI dose 100 times higher than the recommended one showed only minimal side effects assumingly in soils smooth besides the clay and humus contents, which influence the NI diffusion through the soil body, the composition of the nitrifying community and the temperature-related degradation rate whereby DMPP degrades slower than DCD play their role (Azam et al. 2001; Barth et al. 2001; Singh and Verma 2007; Ali et al., 2008; Kleineidam et al., 2011; Mahmood et al., 2011, Tindaon et al., 2011; 2012; 2013; 2019). The latter aspects are also well documented for the most widely used inhibitor nitrapyrin (Ruser et al., 2015).

The NOEL value, calculated based on the recommended dosage of 0.36 (DMPP), 0.25 (ClMPP), and 10 µg g-1 dry soil (DCD) by the USEPA equation, is out of safety considerations divided by 10 (laboratory trials) and 100 (field trials). From our study where DHA (Tab. 3) the NI concentration in the stabilized and granulated N fertilizer must be around 50-100 times higher than the recommended concentration for field application before NOEL started to be surpassed, thus based on our findings and the above discussion and though a risking of side effects cannot fully be excluded a threatening of agroecosystems through the 2 marketed NI DMPP and DCD in the recommended application rate is acceptably safe at our present knowledge.

Dose- Response Relationship

Generally, there is no clear toxicity difference between each nitrification inhibitors, due to NOEL, ED10 and ED50-values. Based on response average values, it can be concluded that ClMPP has more potential side effect on the activities of non target microbes in the soil. This is apparently caused by the effect of halogen element, like chlor, that effectively affects the

Vol. 10, No. 02; 2025

ISSN: 2456-8643

microbial activity in the soil (Meena et al, 2020). Based on the NOEL value, the use of these inhibitors on the dose of 100 times of the recommended dose does not negatively affect the soil environment. All three inhibitors affect non target microbial activities in sandy soils more effectively than in loamy or clayey soils. This is due to the influence of soil clay fraction content that plays a role in adsorption mechanism of inhibitors on the clay surface (Barth et. al., 2001). Environmental risk threshold value was studied based on NOEL equation (Kumar, et al.,2024), that for laboratory trials the average NOEL-value was divided by 10 and for field trials the average NOEL value was divided by 100 (Table 3). It turned out that the environmental risk threshold value is still far above the value of 1-50 times N fertilizer recommended dose (inhibitor incorporated with N fertilizer). This means that the use of these three inhibitors is environmentally compatible and safe.

Table 3. NOEL assessment, ED10 and ED 50 for the three inhibitors in relation with dehydrogenase in three types of clay, loam and sandy based on equations of mathematical models.

Parameters	Soil Type	Ecotoxicological Value for					
		DMPP NOEL ED50	ED_{10}	ClMPP NOEL ED_{50}	ED_{10}	DCD NOEL ED_{50}	ED_{10}
DHA	Clay	91	133	32	66	844	1754
	Loam	371		255		6940	
	Sand	30	72	28	58	550	1126
	Ø	312		229		5558	
		25	56	12	33	167	809
		230		147		4450	
		49	87	24	52	520	1230
		304		210		5649	

Ø[1)] = Average of DHA

4. CONCLUSIONS

The results can be summarizes as follows:

1) The dose response-curves for DHA, DRA and PDC were generally of sigmoid nature in all investigated soils. The dose response curves recorded suggest that DMPP, ClMPP and DCD may affect non target microbial soil processes only at high concentrations.
2) In general, no side effects of the NIs on parameters DHA was observed if rates about 50-100 times the base concentrations, corresponding to 24 µg ClMPP, 49 µg DMPP and 520 µg DCD g^{-1} dry soil were applied (NOEL-value).
3) Dose response relationships between NIs and microbial non target activities depend on soil types. The NOEL, ED10 and ED50-values much higher in clay than in loamy sand or sandy soil. The NIs was generally the most effective in sandy soils.

In conclusion, these three standard methods were reliable and suitable to investigate the side effects of agrochemicals on soil microbial activities in soil because of their reproducibility especially the sensitivity of the methods.

Vol. 10, No. 02; 2025

ISSN: 2456-8643

REFERENCES

Ahmad, M. F., Ahmad, F. A., Alsayegh, A. A., Zeyaullah, M., AlShahrani, A. M., Muzammil, K., ... & Hussain, S. (2024). Pesticides impacts on human health and the environment with their mechanisms of action and possible countermeasures. Heliyon.

Ali R, Iqbal J, Tahir GR, Mahmood T, Pak J (2008) Effect of 3,5-dimetylpyrazole and nitrapyrin on nitrification under high soil temperature. Pakistani Journal of Botany 40: 1053–1062.

Allison, S,D, Claudia, I, . Czimczik,W and Tresder, K.K, 2008. Microbial activity and soil respiration under nitrogen addition in Alaskan boreal forest. Global Change Biology (2008) 14, 1–13, doi: 10.1111/j.1365-2486.2008.01549.x

Araújo, A. S., Leite, L. F., Santos, V. B., & Carneiro, R. F. 2009. Soil microbial activity in conventional and organic agricultural systems. Sustainability, 1(2), 268-276.

Austin A.T, Sala, O.E, and Jackson R.B, 2006: Inhibition of nitrification alters carbon turnover in the Patagonian steppe. *Ecosystems* 9, 1257-1265

Azam, F,. Benckiser, G, Muller, C and Ottow, J.C.G (2001) Release, movement and recovery of 3-4 dimethylpyrazole phosphate (DMPP), ammonium and nitrate from stabilized fertilizer granules in a silty clay soil under laboratory conditions. Biol. Fertil. Soils 34: 118-125

Baldrian,P., 2009. Microbial enzyme-catalyzed processes in soils and their Analysis. Plant Soil Environ., 55,(9): 370–378

Bannert A, Mueller-Niggemann C, Kleineidam K, Wissing L, Cao Z, Schwark L, Schloter M 2011 Comparison of lipid biomarker and gene abundance characterizing the archaeal ammonia-oxidizing community in flooded soils. Biol Fertil Soils 47:839–843

Bardon, C., Poly, F., Piola, F., Pancton, M., Comte, G., Meiffren, G., & el Zahar Haichar, F. 2016. Mechanism of biological denitrification inhibition: procyanidins induce an allosteric transition of the membrane-bound nitrate reductase through membrane alteration. *FEMS microbiology ecology*, *92*(5), fiw034.

Barth, G.2008. Effectiveness of 3,4-Dimethylpyrazole Phosphate as Nitrification Inhibitor in Soil as Influenced by Inhibitor Concentration, Application Form, and Soil Matrix Potential. Pedosphere:18: 378-385

Benckiser, G., & Bamforth, S. S. 2010. Role of pathogens, signal recalcitrance, and organisms shifting for ecosystem recuperation. A review. *Agronomy for sustainable development*.

Benckiser, G, Christ, E, Herbert, T., Weiske, A, Blome, J, Hardt, M, 2013: The nitrification inhibitor 3,4-dimethylpyrazole-phosphat (DMPP) - quantification and effects on soil metabolism Plant Soil DOI 10.1007/s11104-013-1664-6

Douma, AC, Polychronaki, E.A, Giourga, C, and Loumu, A. 2005. Effect of fertilizers with the nitrification inhibitor DMPP (3,4 dimethylpyrazolphosphate) on yield and soil quality. Proc. Of the 9th International Conference on Environmental Science and Technology, Rhodes, Greece, 1-3 September 2005

Ferreira,A.S, Dos Santos,M.A.,and G.F. Correa, 2013. Soil microbial response to glucose and phosphorus addition under agricultural systems in the Brazilian Cerrado. Anais da Academia Brasileira de Ciências 85(1):395-403

Fillery I, 2007. Plant-recommended manipulation of nitrification in soil: a new approach to managing N loss? Plant and Soil 294: 1-4

Kisand, V., and Wikner, J. 2003. Combining culture-dependent and-independent methodologies for estimation of richness of estuarine bacterioplankton consuming riverine dissolved

organic matter. *Applied and environmental microbiology*, *69*(6), 3607-3616. doi: 10.1128/AEM.69.6.3607-3616.2003

Kleineidam, K., Košmrlj, K., Kublik, S., Palmer, I., Pfab, H., Ruser, R., ... & Schloter, M. (2011). Influence of the nitrification inhibitor 3, 4-dimethylpyrazole phosphate (DMPP) on ammonia-oxidizing bacteria and archaea in rhizosphere and bulk soil. *Chemosphere*, *84*(1), 182-186.

Kumar, A., Ojha, P. K., & Roy, K. (2024). Chemometric modeling of the lowest observed effect level (LOEL) and no observed effect level (NOEL) for rat toxicity. Environmental Science: Advances, 3(5), 686-705.

Liao, M., Luo, Y. K., Zhao, X. M., & Huang, C. Y. (2005). Toxicity of cadmium to soil microbial biomass and its activity: effect of incubation time on Cd ecological dose in paddy soil. Journal of Zhejiang University-Science B, 6(5), 324-330.

Mahmood T, Ali R, Latif Z, Ishaque W (2011). Dicyandiamide increases the fertilizer N loss from an alkaline calcareous soil treated with 15N-labelled urea under warm climate and under different crops. BiologyandFertilityofSoils 147: 619–631.

Meena, R. S., Kumar, S., Datta, R., Lal, R., Vijayakumar, V., Brtnicky, M., ... & Marfo, T. D. (2020). Impact of agrochemicals on soil microbiota and management: A review. Land, 9(2), 34.

Moreno, J. L., Garcıa, C., Landi, L., Falchini, L., Pietramellara, G., & Nannipieri, P. (2001). The ecological dose value (ED50) for assessing Cd toxicity on ATP content and dehydrogenase and urease activities of soil. Soil Biology and Biochemistry, 33(4-5), 483-489.

O'Callaghan M, Gerard EM, Carter PE, Lardner R, Sarathchandra U, Burch G, Ghani A, B ell N , 2010. Effect of the nitrification inhibitor dicyandiamide (DCD) on microbial communities in a pasture soil amended with bovine urine. Soil Biol. Biochem. 42(9): 1425-1436

PICCMAT (2011). Politic Incentives for Climate Change Mitigation Agricultural Techniques for an agricultural policy towards the year 2020 http://www.climatechangeintelligenceaastel.be/ iccmat/index.php

Quilchano C. and Maranon T., 2002. Dehydrogenase activity in Mediterranean forest soils. Biol. Fert. Soils, 35, 102-107.

Rahmatpour, S., Shirvani, M., Mosaddeghi, M. R., Nourbakhsh, F., & Bazarganipour, M. (2017). Dose–response effects of silver nanoparticles and silver nitrate on microbial and enzyme activities in calcareous soils. *Geoderma*, *285*, 313-322.

Ruser, R., & Schulz, R. (2015). The effect of nitrification inhibitors on the nitrous oxide (N2O) release from agricultural soils—a review. Journal of Plant Nutrition and Soil Science, 178(2), 171-188.

Sahrawat, K.L, 2004. Nitrification inhibitors for controlling methane emission from submerged rice soils. Current Science. 87:1084-1087

Šantric,L.L; Radivojević, L., Umiljendić,J.G, Đurović-Pejčev, R., and M. Sarić-Krsmanović 2014. Assessment of microbial activity and biomass in different soils exposed to nicosulfuron. Pestic. Phytomed. (Belgrade), 29(3), 2014, 213–219 . DOI: 10.2298/PIF1403213S

Singh. S. N. and Verma A., (2007). The Potential of Nitrification Inhibitors to Manage the Pollution Effect of Nitrogen Fertilizers in Agricultural and Other Soils: A Review Environmental Practice 9:266–279

Tindaon, F., Benckiser, G., & Ottow, J. C. (2011). Toxicity of Nitrification Inhibitors on Dehydrogenase Activity in Soils. *International Journal on Advanced Science, Engineering and Information Technology*, *1*(1), 98-103.

Tindaon, F., Simarmata, T., Benckiser, G., & Ottow, J. C. G. (2011). by Azospirillum brasilense and Sinorhizobium sp in the presence of the nitrification inhibitors. *Bionatura*, *13*(3).197-208

Tindaon, F., Benckiser, G., & Ottow, J. C. G. (2011). Effects of nitrification inhibitors on mineral nitrogen dynamics in agriculture soils. *AGRIVITA, Journal of Agricultural Science*, *33*(3), 279-290

Tindaon, F., Benckiser, G., & Ottow, J. C. (2012). Evaluation of ecological doses of the nitrification inhibitors 3, 4-dimethylpyrazole phosphate (DMPP) and 4-chloromethylpyrazole (ClMP) in comparison to dicyandiamide (DCD) in their effects on dehydrogenase and dimethyl sulfoxide reductase activity in soils. *Biology and fertility of soils*, *48*(6), 643-650.

Tindaon, F., Benckiser, G., & Ottow, C. G. (2013). Side effects of nitrification inhibitors on non target microbial processes in soils. *Journal of Tropical Soils*, *16*(1), 7-16.

Tindaon F, Benckiser, G. 2019. Evaluation of the Side Effects of Nitrification-Inhibiting Agrochemicals in Soils. In: Sayyed R., Reddy M., Antonius S. (eds) Plant Growth Promoting Rhizobacteria (PGPR): Prospects for Sustainable Agriculture. Springer, Singapore. https://doi.org/10.1007/978-981-13-6790-8_6\

Welp, G., & Brümmer, G. W. (1997). Toxicity of increased amounts of chemicals and the dose–response curves for heterogeneous microbial populations in soil. *Ecotoxicology and environmental safety*, *37*(1), 37-44.

Zanatta, J. A., Bayer, C., Dieckow, J., Vieira, F. C. B., & Mielniczuk, J. 2007. Soil organic carbon accumulation and carbon costs related to tillage, cropping systems and nitrogen fertilization in a subtropical Acrisol. *Soil and Tillage Research*, *94*(2), 510-519.

Zerulla, W., Barth, T., Dressel, J, Erhardt, K., Horchler von Loqueqhien, K, Pasda, G., Raedle, M and Weissmeier, A.H (2001) . 3-4 dimethylpyrazole phosphate (DMPP) – a new nitrification inhibitor for agriculture and horticulture. An Introduction. Biol. Fertil. Soils: 34: 79-84.