Effect of pharmaceutical micropollutants (Acetaminophen and Hydroxychloroquine) and its degradative products on soil nitrate

M.S. Waghmode[1]*, P.S. Abhyankar [2] and N.N. Patil[1]

[1]Department of Microbiology, PDEA's Annasaheb Magar Mahavidyalaya (Affiliated to Savitribai Phule Pune University), Hadapsar, Pune-411028, Maharashtra, India.
[2]Department of Microbiology, The P.G.K.Mandal H. V. Desai College (Affiliated to Savitribai Phule Pune University), Pune-411002, Maharashtra, India.

Date Received: 21-09-2024 Date Accepted: 25-12-2024

Abstract

Pharmaceutical drugs and its degradative products have been reported in the environmental samples like soil, sediment, and water. Based on the limit of detection very few studies have been conducted on the effect of persistent, bio accumulative and toxic drugs on the environment. Considering the impact of nitrate on soil fertility and vegetables, current study was done to assess the impact of acetaminophen, hydroxychloroquine, acetaminophen biodegradative products (hydroquinone, 4 Aminophenol), and hydroxychloroquine biodegradative products (7 Chloroquinoline 4 amine, oxalic acid) on the soil nitrogen transformation. The test compounds (10 mg/kg, 100 mg/kg, and 1000 mg/kg) were added to soil samples, whereas untreated samples served as the control. Samples of treated and control soils were processed for nitrate estimate using the Mgo-Devarda's alloy technique for nitrate-N estimation after 0, 7, 14, and 28 days of incubation. Pollutants found to have significant effect on soil nitrate. At high concentration (1000 mg/kg), soil nitrate concentration was in the extremely high region suggesting possible impact on the soil fertility and accumulation in vegetable parts. Vegetable nitrate content is considered as important quality parameter for human health. This data is helpful for determination of the negative impact of the pharmaceuticals on human diet and subsequently suggesting for the environmental monitoring of drugs before discharge into the ecosystem.

Keywords: *Acetaminophen, 4-Aminophenol, Hydroquinone, Hydroxychloroquine, Nitrate, Soil.*

**Correspondence: meghmicro2@gmail.com*

1. Introduction:

Excessive usage of pharmaceuticals and personal care products are leading to the environmental contamination, antimicrobial drug resistance in pathogens and bioaccumulation in food crops (Waghmode et al., 2023). One important microbiological process in soil that is vital to preserving soil fertility is the mineralization of nitrogen in organic materials. It is vital to consider the possible impact of a substance on the microbiological activity of the soil when conducting a risk assessment for a material that might get into the soil environment (Roberts et al., 2010).

The percentage of pollutants that are bioavailable usually has a greater influence on the environment than the total amounts of pollutants in the soil (Alexander, 2000). Using "mild" reagents (such as 0.01 mol L−1 CaCl2 solution), some loosely attached pollutants can be removed (Barriuso et al., 2004). By using more powerful extraction procedures, the more firmly bonded contaminant in the soil can subsequently be extracted from another nonextractable fraction (Stokes et al., 2006).

In the soil, the presence of Metronidazole, Tetracycline, Chlortetracycline47, Tylosine, Sulfadimethoxine and Flumequine has been reported (Díaz-Cruz et al., 2003), whereas Estriol, Estradiol, Ethynyl estradiol, Estrone Diethylstilbestro, Norethindrone, Levonorgestrel and Progesterone have been reported to be present in water samples (Díaz-Cruz et al., 2003). Reactive oxygen and nitrogen species are produced from endogenous sources (NADPH, endoplasmic reticulum, mitochondria, xanthine oxidases) and exogenous sources (recalcitrant compounds, dyes, heavy metals, drugs, radiations) (Aranda-Rivera et al., 2022). Acetaminophen, 4 Aminophenol, Hydroxychloroquine, 7 chloroquinoline-4 amine and 4 aminoquinoline, contains 'N' atom in their structure, thereby generating both reactive oxygen and reactive nitrogen species (Humayun et al., 2023). Hydroquinone, 1,4 Benzoquinone, and oxalic acid generates reactive oxygen species. Reactive oxygen and nitrogen species imposes the oxidative stress on the organisms which activates the lytic enzymes involved in the programmed cell death (Sigler et al., 1999).

Pharmaceutical goods can negatively affect the nitrogen cycle in both aquatic and terrestrial ecosystems, which can influence crop nutrition, soil fertility, and other nitrogen-related changes in our environment. For example, it has been demonstrated that fluoroquinolones and sulfonamides partially inhibit denitrification in the environment (Rosendahl et al., 2012). The environmental risks of fluoroquinolone antibiotic was assessed using nitrogen level as parameter. Difloxacin antibiotic is persistent drug with dissipation half-life of more than 217 days in bulk soil. But it showed very limited effect on nitrogen turnover due to the drug's strong binding and less bio accessibility (Rosendahl et al., 2012).

Most of the nitrogen in the soil is found in organic compounds, which are necessary for plant growth. However, plants are unable to directly use organic nitrogen since it must first be transformed into ammonium or nitrate ions to be absorbed (Gaskell, and Smith., 2007). The nitrogen cycle is the aggregate term for the several environmental nitrogen transformations that include nitrogen fixation, assimilation, nitrification, and denitrification.

Nitrogen transformations are crucial to the sustenance of living things and microbes. Therefore, nitrogen plays a key role in controlling the biosphere's primary production (Gruber and Galloway, 2008). However, nitrogen from fertilizers and manures are readily lost to the environment and has a variety of detrimental effects to the sustainable management of land, air, and water. The most significant dietary source of nitrates for humans is vegetables. Increased nitrate levels in vegetables may cause several illnesses, including cancer and blue baby syndrome, because of nitrites, which are nitrate's metabolically altered byproduct (Salehzadeh et al., 2020).

Accumulation of the nitrate and oxalic acid, negatively affect the nutritional contents and antioxidant properties of the plants like purslane (*Portulaca oleracea*) (Santos et al., 2014).

Nitrate content in the vegetables, depends upon growing conditions of the vegetables, harvesting season, irrigation water, nitrogenous fertilizers, and type of vegetable (Salehzadeh et al.,

*Correspondence: meghmicro2@gmail.com

2020). Hence different standards like European Commission Regulation (No. 194/97), has been proposed to regulate and monitor the concentration of the nitrate in the vegetables.

Acetaminophen and Hydroxychloroquine, were extensively used and emergent micropollutants during as well as post Covid 19 pandemic situation (Waghmode et al., 2022). Various physico-chemical and biological methods have been used for the detoxification of the water reservoirs polluted with pharma pollutants (Waghmode et al.,2024).

The sorption/desorption of pharmaceutical residues in soil depends on factors like the physicochemical properties of the compound, and environmental properties of the soil (Pérez-Lucas, and Navarro, 2024). Determining microbial responses under exposure conditions can assist in elucidating the impact of chemicals on soil microbial activity and community size which subsequently affects the soil remediation (Pérez-Lucas, and Navarro, 2024).

2 Materials and Methods:

2.1 Physicochemical properties of the experimental soil:

Soil sampling was done from a nearby farm, located in the rural part of Pune city, Maharashtra state, India. Agricultural soil was collected from the surface plough (15 cm) with a soil auger to a depth of 15 cm with a spade. A uniform slice of soil, up to 4 to 6 cm thick, parallel to the V shaped cut was taken. Sieving was done to remove the pebbles, and stones.

The soil physicochemical properties were determined before experimentation. Soil p^H and conductivity was determined using Equiptronics p^H meter and electric conductivity meter (NOEQ 660A, India) (Karastogianni et al., 2016). The bulk density of soil was determined using the method APHA, 2017. Five gram of soil sample was taken in a dry empty bottle with intermittent tapping for 5-10 times, and the weight of the bottle was measured. Bulk density of the soil was calculated using Eq 1.

$$\text{Bulk density}\left(\frac{\text{g}}{\text{cm}^3}\right) = \frac{\text{M}_2-\text{M}_1}{\text{V}} \qquad (1)$$

where, M_2 is the weight of the bottle containing soil; M_1 is the weight of empty bottle; V is the volume. Using Equations 2 and 3, the soil porosity was assessed by adding water after tapping five to ten times until the soil was submerged. The amount of water (millilitre) was then estimated.

$$\text{Pore Space} = \text{Volume of soil and water} - \text{Volume of water} \qquad (2)$$

$$\text{Porosity}\left(\frac{\text{Particle}}{\text{Volume}}\right) = \frac{\text{Pore space}}{\text{Total volume of soil and water}} \qquad (3)$$

2.2 Artificial contamination of soil:

The OECD, 216 protocol was followed in the preparation of soil samples. The test substances were added to sieved soil at concentrations of 10 mg/kg, 100 mg/kg, and 1000 mg/kg, whereas the control was left untreated. The nitrate estimate was performed on samples of treated and control soils after 0, 7, 14, and 28 days of incubation at room temperature. Three sets of each chemical were made.

2.3 Estimation of Nitrate N:

Soil nitrate was determined using the Mgo-Devarda’s alloy method at Mahatma Phule Agricultural University, Pune (Bremner and Keeney, 1965). Kelplus automatic nitrogen analyser was used for the study. For the evaluation of soil nitrate content, 5 g of sieved soil was added to a micro Kjeldahl flask containing 0.2 g of Devarda's alloy. The stopper was placed to let the steam out. Addition of 25 mL of the 2N KCl solution, and 0.2 g of MgO was done. The micro-Kjeldahl flask was attached to the condenser containing boric acid indicator solution. Once the distillate reaches to the 30 ml mark on the beaker, steam was turn off. The collected distillate was titrated by using 0.005N H_2SO_4 standard. The end point was determined based on the colour changes from green to a permanent pale pink. Blank was run without soil. Nitrate nitrogen was calculated using the Eq.4 (Liao,1981).

$$\%\text{ of NO}_3 - \text{N} = (\text{A} - \text{B}) \times \text{Normality of Sulfuric Acid} \times 0.014 \times \frac{100}{\text{Weight of sample (g)}} \quad (4)$$

*Correspondence: meghmicro2@gmail.com

where A is the volume of H_2SO_4 (in ml) required for soil with Devarda's alloy distillation and B is the volume of H_2SO_4 (in ml) required for blank distillation with Devarda's alloy distillation. Nitrate was calculated using Eq.5 (Liao,1981).

$$Nitrate\ \left(\frac{Kg}{Ha}\right) = \%\ of\ Nitrate\ \times 22400 \qquad (5)$$

The experiment was done in triplicates. Microsoft excel 2019 was used for the determination of the average reading and standard error of mean.

3. Results and Discussion:

Pharmaceutical drugs have been reported in the soil and water, where they will affect the nitrogen cycle (Pashaei et al., 2022). At present, both industrialised and developing nations have large populations of soils contaminated with pollutants; risk assessment and remediation of these sites are critical. The variety of toxicants and the historical character of the pollution can make containment and remediation solutions more difficult. There is need to study about the behaviour of the pollutants in soil and the variables that influence. Understanding the bioavailable fraction and the overall concentration of contaminants in soil is essential because it plays a crucial role in toxicity and biodegradation (Stokes et al., 2006). Quantifying the bioavailable fraction should also be done whenever feasible. For studying the environmental fate of the drugs, their degradative pollutants in the soil, data related to the physicochemical properties of the soil, pollutants, extractable residue, and half-life are needed. The biodegradation pathway of Acetaminophen and Hydroxychloroquine is given in Figures 1 and 2 respectively.

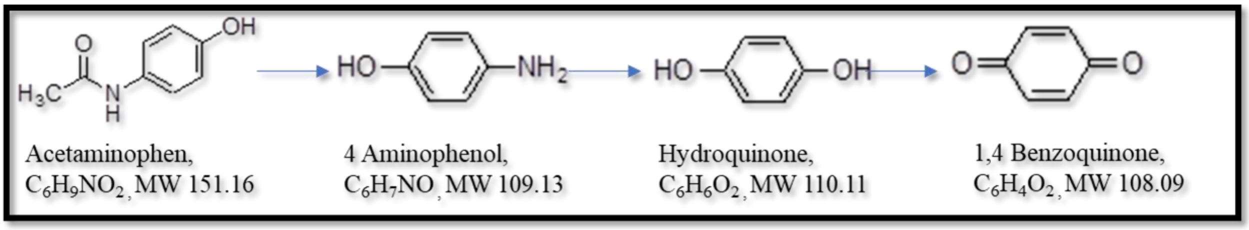

Figure 1. Acetaminophen Biodegradative pathway by 4 Aminophenol pathway (Adapted from Hu et al., 2013)

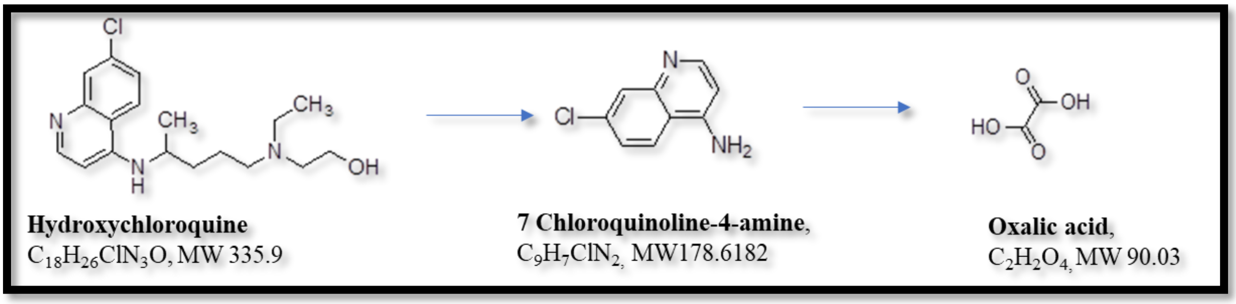

Figure 2. Hydroxychloroquine Biodegradative pathway by *Kosakonia cowanii* JCM 10956(T) (Adapted from Waghmode *et al*., 2023).

Hydroquinone, 4 Aminophenol and 1,4 Benzoquinone are the main degradative products by bacteria and fungi (Hu et al., 2012; Waghmode and Patil, 2023). For hydroxychloroquine, 7 Chloroquinoline 4 amine, 4 aminoquinoline-7-ol and oxalic acid has been reported as the degradative products by electrochemical advanced oxidation processes (Bensalah et al., 2020), biodegradation mediated by *Kosakonia cowanii* JCM 10956(T) (Waghmode et al., 2023) and *Bacillus velezensis* (Waghmode and Patil, 2023). The current study focused on evaluating the role of the pharmaceutical micropollutants and their reported degradative products on the soil nitrate content.

*Correspondence: meghmicro2@gmail.com

3.1 Physicochemical properties of the experimental soil:

Experimental soil was tested for physicochemical properties like pH, bulk density (g/cm3), porosity(particle/volume), and electric conductivity (mS/m). pH of the soil was found to be 7.01 which suggests the neutral nature of the soil. Bulk density and porosity were found to be 0.067 g/cm^3 and 0.4477 particle/volume. Electrical conductivity of the soil was found to be 35 mS/m. A soil's bulk density is a dynamic attribute that changes depending on the structural conditions of the soil, organic matter concentration, porosity, and compaction (Chaudhari et al., 2013). Salinity and soil water content are commonly associated with soil electrical conductivity (Brevik et al., 2006). The electrical conductivity indicated that the soil had a low salinity.

3.2 Artificial contamination of soil:

Soil samples were artificially polluted with the test samples as per the protocol prescribed by OECD, 216.Sieved soil was spiked with the test substance (10 mg/kg; 100 mg/kg;1000mg/kg) and untreated as control.

3.3 Estimation of Nitrate N:

After 0, 7, 14 days and 28 days of incubation, samples of treated and control soils processed for the nitrate estimation. Soil was treated with KCl to remove nitrogen and ammoniacal nitrogen. By treating the soil successively with MgO and Devarda's alloy, evolved ammonia was collected in boric acid to produce ammonium borate, which was used to calculate the ammonium-nitrogen (NH_4-N) and nitrate -nitrogen (NO_3-N). The ammonium borate was titrated using standard H_2SO_4, and the amounts of NH_4-N and NO_3-N needed were computed based on the volumes of standard H_2S0_4. Interpretation of the soil nitrate was done as low (<281Kg/Ha), normal (281-420 Kg/Ha), moderately high (421-750 Kg/Ha), high (750-1000 Kg/Ha) and very high (>1000 Kg/Ha). It was discovered that the experimental control soil had a low nitrate concentration. After exposing the soil to the parent drugs (Acetaminophen and Hydroxychloroquine) and its degradative products (4 Aminophenol, Hydroquinone, 4 Amino 7 Chloroquinoline, and Oxalic acid obtained results are given in bar diagram as Figures 3-8.

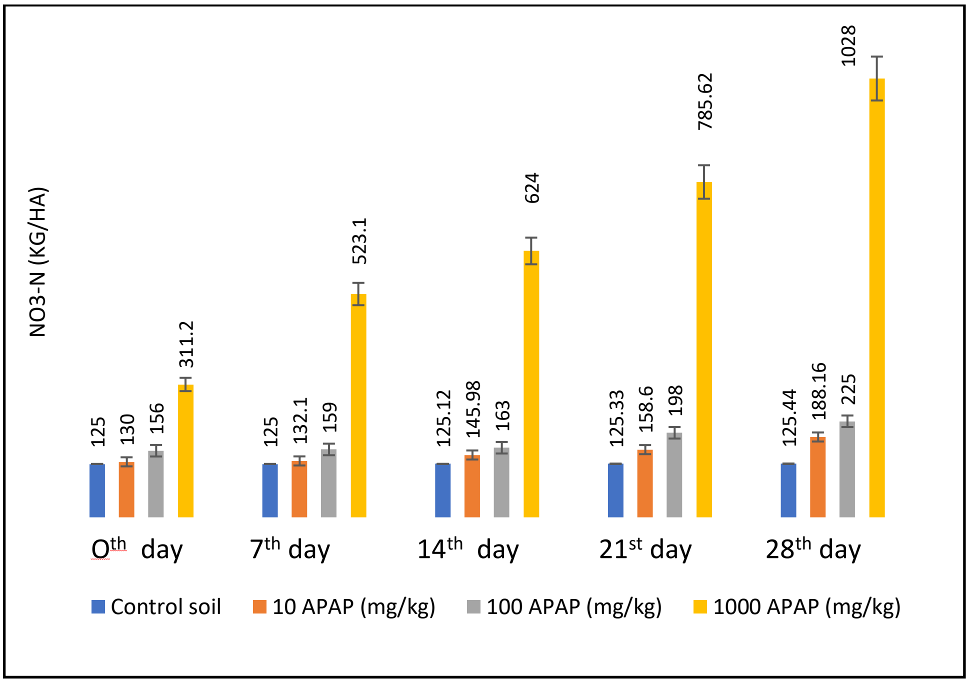

Figure 3. Effect of Acetaminophen (10, 100, 1000 Mg/Kg) on soil nitrate.

*Correspondence: meghmicro2@gmail.com

At concentrations higher than 250 parts per million, acetaminophen slowed down the rate of nitrification and denitrification in water (Caracciolo et al., 2015). As seen in Fig. 3, acetaminophen significantly raised the amount of nitrate in the soil subjected to 1000 mg/kg for 28 days throughout this investigation. Quinone-like compounds (hydroquinone and p-benzoquinone) inhibits nitrification by obstructing the activity of the ammonia oxidising bacteria (AOB) with IC50 values at 1 h of exposure time of 3.1 ± 0.5 ppm, and 2.8 ± 0.4 ppm (Suárez-Ojeda *et al*., 2010). At the concentration of 1000 mg/kg , hydroquinone showed increase in the nitrate content as shown in Fig.4.

One of the chemicals that is frequently found in both biological and cosmetic sources is hydroquinone (Valenzuela et al., 2024). According to reports, hydroquinone imposes ecotoxicity by suppressing the microbial communities and their metabolic functioning (Enguita, and Leitão, 2013). According to the available report, at 100 µg/ml, hydroquinone has a substantial impact on the microbial development and metabolic capability of the soil and river microbial communities (Valenzuela et al., 2024). Acetaminophen degradative product 4 aminophenol has a more detrimental effect on the soil nitrate level than the parent medications acetaminophen and hydroquinone as shown in Fig.5. Following 28 days of soil exposure to 4 aminophenol, the nitrate was discovered to be in the extremely high category. The amino (NH_2-) group in the four aminophenol has an impact on the pace at which nitrogen is transformed in the soil.

In the current study, the drugs and their degradative products containing amino group (4 Aminophenol) showed significant increase in the soil nitrate content which may impose the risk of soil nonfertility.

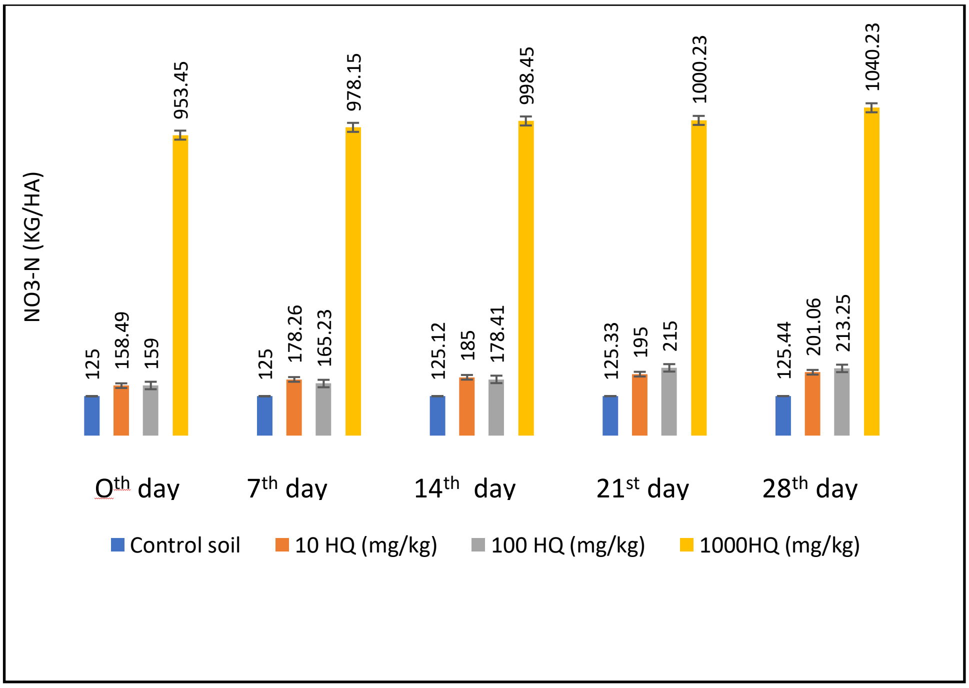

Figure 4. Effect of Hydroquinone (10, 100, 1000 Mg/Kg) on soil nitrate

*Correspondence: meghmicro2@gmail.com

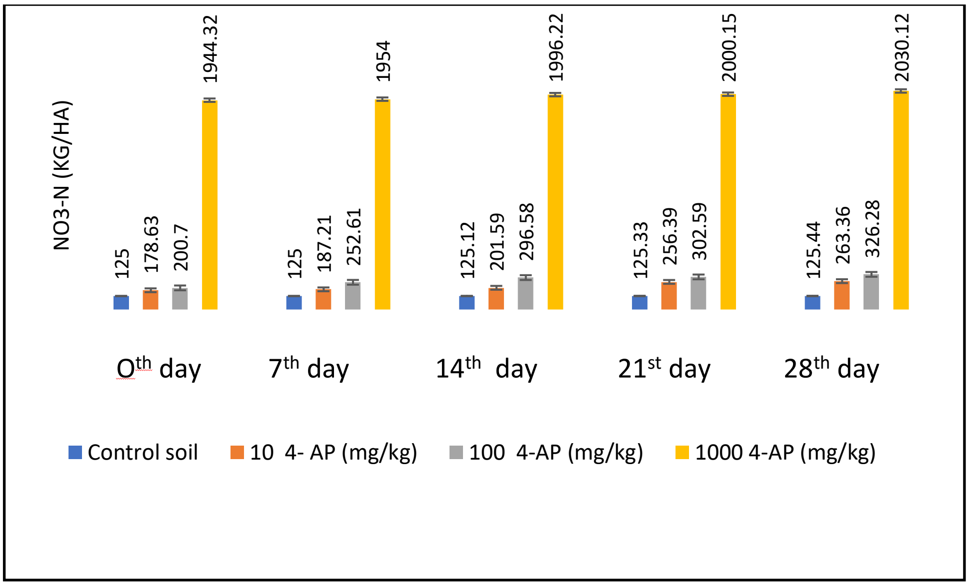

Figure 5. Effect of 4- Aminophenol (10, 100, 1000 Mg/Kg) on soil nitrate.

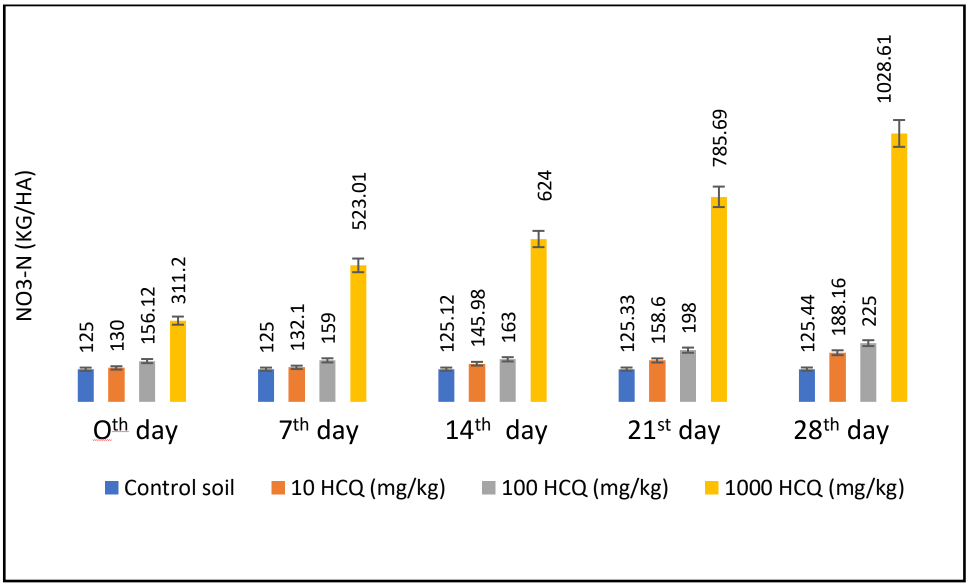

Figure 6. Effect of Hydroxychloroquine (10,100, 1000 Mg/Kg) on soil nitrate.

By generating hydroxyl radicals, nitrate has been identified as a crucial component in the increased photodegradation of hydroxychloroquine. Therefore, the nitrate level in the soil that was spiked with hydroxychloroquine only significantly increased (Figure .6). The obtained results are in accordance with the data of Dabic et al., 2019.

Depending on the period and dosage, the degradative product of hydroxychloroquine, oxalic acid, demonstrated a greater rise in the soil nitrate level (Fig. 6,7,8). This result is in line with the previously published data on Spinach leaves (Logegaray and Frezza,2023).

Due to potential health effects, governments and regulators are interested in vegetable nitrate levels and want to make sure content regulations are working. Nitrate has few harmful impacts on health when taken alone, but its metabolites can have a variety of negative effects. Up until recently, nitrate was thought to be a purely dangerous food ingredient that causes carcinogenesis, infantile hemoglobinemia, and probably even teratogenesis (Santamaria, 2006).

*Correspondence: meghmicro2@gmail.com

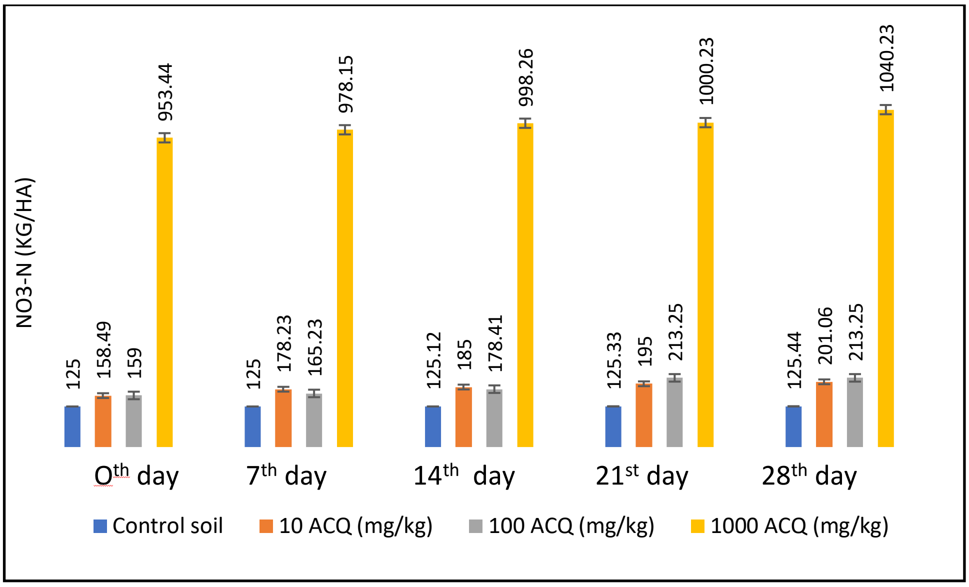

Figure 7. Effect of 4 Amino 7 Chloroquinoline (10,100, 1000 Mg/Kg) on soil nitrate

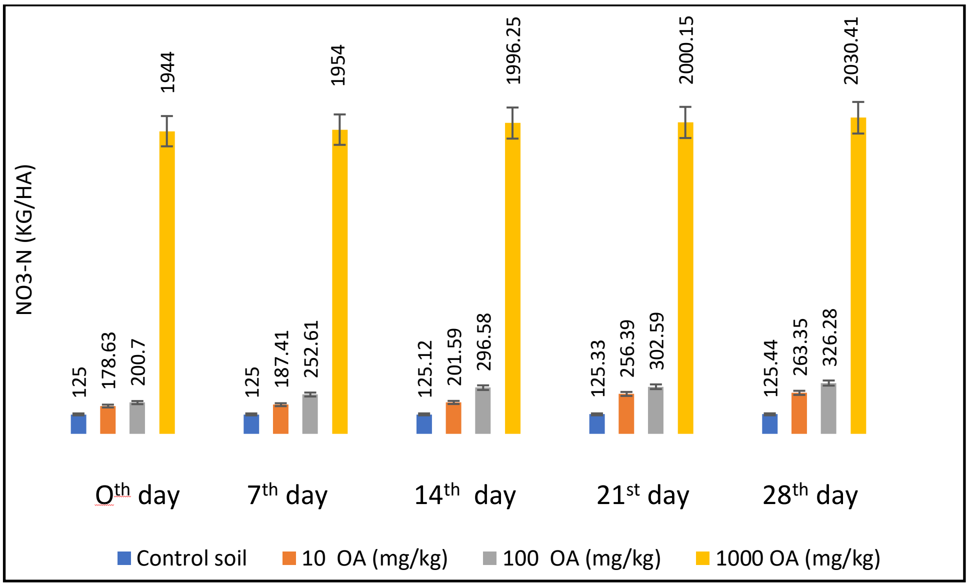

Figure 8. Effect of Oxalic acid (10,100, 1000 Mg/Kg) on soil nitrate.

All the test substances (Acetaminophen, 4 Aminophenol, Hydroquinone, Hydroxychloroquine, 7-chloroquinoline-4-amine, oxalic acid) showed extensive increase in the nitrate contents at the concentration of 1000 mg/Kg. Based on the soil adsorption coefficient (Koc) values, acetaminophen (Koc 21), 1,4 benzoquinone (Koc 30), hydroquinone (Koc 50), oxalic acid (Koc 5) if released to soil it can get leach and may volatilize or photodegrade on soil surfaces (Pan and Chu, 2016). The impact of this chemicals on crops can be reduced by natural degradation of these chemicals. The rate at which nitrogen is transformed in soil may be impacted by negative impact of the chemicals on the native microbial population.

4. Conclusions

Emerging pharmaceutical micropollutants can have direct as well as indirect effects on the non-target organism. Less research has been done on the environmental impact of hazardous,

*Correspondence: meghmicro2@gmail.com

bioaccumulative, and persistent medicines and their degradative byproducts. Crop production and human life are both impacted by high levels of nitrate residue in the soil and water. The goal of this study was to determine how the medications and their breakdown products affected the amount of nitrate in the soil when combined at varying concentrations (10, 100, and 1000 mg/kg). Since vegetables account for up to 80% of nitrate intake in the human diet, it is important to closely monitor the nitrate concentration while growing vegetables on agricultural land close to pharmaceutical manufacturing facilities.

A thorough investigation of how pharmaceutical contaminants affect the microbial community (involved in nitrogen metabolism), soil physicochemical characteristics, and crop productivity is required. The physicochemical characteristics of the agricultural soil and water must be preserved for crops and vegetables to be healthy.

Acknowledgement: Ms. Meghmala Waghmode acknowledges MahaJyoti Fellowship 2022.

References

Alexander, M.,2000. Aging, bioavailability, and overestimation of risk from environmental pollutants. Environmental science and technology, 34(20), pp 4259-4265. https://doi.org/10.1021/es001069.

APHA. 1998. Standard methods for analysis of water and waste water. The American Public Health Association, United States.

Barriuso, E., Koskinen, W. C., and Sadowsky, M. J.,2004. Solvent extraction characterization of bioavailability of atrazine residues in soils. Journal of agricultural and food chemistry, 52(21), pp 6552-6556. https://doi.org/10.1021/jf040245l.

Bensalah, N., Midassi, S., Ahmad, M. I., and Bedoui, A.,2020. Degradation of hydroxychloroquine by electrochemical advanced oxidation processes. Chemical Engineering Journal, 402, 126279. https://doi.org/10.1016/j.cej.2020.126279.

Bremner, J. M., and Keeney, D. R.,1965. Steam distillation methods for determination of ammonium, nitrate and nitrite. Analytica chimica acta, 32, 485-495.https://doi.org/10.1016/S0003-2670(00)88973-4.

Brevik, E.C., Fenton, T.E. and Lazari, A., 2006. Soil electrical conductivity as a function of soil water content and implications for soil mapping. Precision Agriculture, 7, pp.393-404.https://doi.org/10.1007/s11119-006-9021-x.

Caracciolo, A. B., Topp, E., and Grenni, P.,2015. Pharmaceuticals in the environment: biodegradation and effects on natural microbial communities. A review. Journal of pharmaceutical and biomedical analysis, 106, pp 25-36. https://doi.org/10.1016/j.jpba.2014.11.040.

Chaudhari, P.R., Ahire, D.V., Ahire, V.D., Chkravarty, M. and Maity, S., 2013. Soil bulk density as related to soil texture, organic matter content and available total nutrients of Coimbatore soil. *International Journal of Scientific and Research Publications*, *3*(2), pp.1-8.

Dabić, D., Babić, S., and Škorić, I.,2019. The role of photodegradation in the environmental fate of hydroxychloroquine. Chemosphere, 230, pp 268-277.

Díaz-Cruz, M. S., de Alda, M. J. L., and Barcelo, D.,2003. Environmental behavior and analysis of veterinary and human drugs in soils, sediments, and sludge. TrAC Trends in Analytical Chemistry, 22(6), pp 340-351.

Enguita, F.J. and Leitão, A.L., 2013. Hydroquinone: environmental pollution, toxicity, and microbial answers. BioMed research international, 2013(1), p.542168. https://doi.org/10.1155/2013/542168.

Gaskell, M. and Smith, R., 2007. Nitrogen sources for organic vegetable crops. *HortTechnology*, *17*(4), pp.431-441.

*Correspondence: meghmicro2@gmail.com

Gruber, N., and Galloway, J. N.,2008. An Earth-system perspective of the global nitrogen cycle. Nature, 451(7176), pp 293-296.

Hu, J., Zhang, L. L., Chen, J. M., and Liu, Y. ,2013. Degradation of paracetamol by Pseudomonas aeruginosa strain HJ1012. Journal of Environmental Science and Health, Part A, *48*(7), pp 791-799.

Humayun, S., Hayyan, M., and Alias, Y.,2023. A review on reactive oxygen species-induced mechanism pathways of pharmaceutical waste degradation: Acetaminophen as a drug waste model. Journal of Environmental Sciences.147,pp 688-713 https://doi.org/10.1016/j.jes.2023.11.021.

Karastogianni, S., Girousi S. and Sotiropoulos, S.,2016. pH: Principles and measurement. In: Caballero, B., Finglas, P. and Toldra, F. (eds.) The Encyclopedia of Food and Health. 4; pp 333-338. Oxford: Academic Press.

Liao, C.F., 1981. Devarda's alloy method for total nitrogen determination. *Soil Science Society of America Journal*, *45*(5), pp.852-855.

Logegaray, V.R. and Frezza, D., 2023. Effect of nitrogen fertilization on nitrates and oxalic acid content in spinach leaves (*Spinacia oleracea* L.).

OECD, 2000. Guideline for the Testing of Chemicals No. 216. In: Soil Microorganisms: Nitrogen Transformation Test. Organisation of Economic Cooperation and Development, Paris.

Pan, M. and Chu, L.M., 2016. Phytotoxicity of veterinary antibiotics to seed germination and root elongation of crops. Ecotoxicology and Environmental Safety, 126, pp.228-237.https://doi.org/10.1016/j.ecoenv.2015.12.027.

Pashaei, R., Zahedipour-Sheshglani, P., Dzingelevičienė, R., Abbasi, S., and Rees, R.M., 2022. Effects of pharmaceuticals on the nitrogen cycle in water and soil: a review. Environmental Monitoring and Assessment, 194(2), p.105.

Pérez-Lucas, G. and Navarro, S., 2024. How Pharmaceutical Residues Occur, Behave, and Affect the Soil Environment. *Journal of Xenobiotics*, *14*(4), pp.1343-1377. https://doi.org/10.3390/jox14040076.

Roberts, G., Penwell, A., Peurou, F., and Sharpe, A.,2010. The effect of soil moisture content on nitrogen transformation using OECD test guideline 216. *Applied soil ecology*, *46*(3), pp 478-482.

Rosendahl, I., Siemens, J., Kindler, R., Groeneweg, J., Zimmermann, J., Czerwinski, S., and Amelung, W. ,2012. Persistence of the fluoroquinolone antibiotic difloxacin in soil and lacking effects on nitrogen turnover. Journal of environmental quality, 41(4), pp 1275-1283. https://doi.org/10.2134/jeq2011.0459.

Salehzadeh, H., Maleki, A., Rezaee, R., Shahmoradi, B., and Ponnet, K.,2020. The nitrate content of fresh and cooked vegetables and their health-related risks. PloS one, 15(1), e0227551, https://doi.org/10.1371/journal.pone.0227551.

Santamaria, P.,2006. Nitrate in vegetables: toxicity, content, intake and EC regulation. Journal of the Science of Food and Agriculture, 86(1), pp 10-17.https://doi.org/10.1002/jsfa.2351

Santos, R. V., Machado, R. M. A., Alves-Pereira, I., and Ferreira, R. M. A.,2014. The influence of nitrogen fertilization on growth, yield, nitrate and oxalic acid concentration in purslane (Portulaca oleracea). In VI Balkan Symposium on Vegetables and Potatoes 1142 (pp. 299-304).

Sigler, K., Chaloupka, J., Brozmanova, J., Stadler, N. and Höfer, M., 1999. Oxidative stress in microorganisms—I: microbial vs. higher cells—damage and defenses in relation to cell aging and death. Folia Microbiologica, 44, pp.587-624.

Stokes, J. D., Paton, G. I., and Semple, K. T. ,2005. Behaviour and assessment of bioavailability of organic contaminants in soil: relevance for risk assessment and remediation. Soil Use and Management, 21, pp 475-486. https://doi.org/10.1079/SUM2005347.

**Correspondence: meghmicro2@gmail.com*

Suárez-Ojeda, M. E., Guisasola, A., and Carrera, J.,2010. Inhibitory impact of quinone-like compounds over partial nitrification. Chemosphere, 80(4),pp 474-480. https://doi.org/10.1016/j.chemosphere.2010.03.041.

Valenzuela, A., Ballestero, D., Gan, C., Lorca, G., Langa, E., and Pino-Otín, M. R.,2024. Hydroquinone Ecotoxicity: Unveiling Risks in Soil and River Ecosystems with Insights into Microbial Resilience. Toxics, 12(2), 115. https://doi.org/10.3390/toxics12020115.

Waghmode Meghmala, Patil Neha, Gaikwad Dattatray.,2023. Bioremediation of Acetaminophen and Hydroxychloroquine by Kosakonia cowanii JCM 10956(T) with ecotoxicity studies, PREPRINT (Version 1) available at Research Square https://doi.org/10.21203/rs.3.rs-2940886/v1.

Waghmode, M. S., Patil, N. N., and Bankar, A. V.,2022. Microbial Cell Factories For the Management of Pharmaceutical Micropollutants. International Journal of Ecology and Environmental Sciences, 49(1),pp 5-16.

Waghmode, M. S., Gunjal, A. B., and Patil, N. N. ,2023. Social Management of Pharma Products for Sustainable Development. In Positive and Constructive Contributions for Sustainable Development Goals (pp. 181-199). IGI Global.

Waghmode. and Patil N., 2023. Bioremediation of Acetaminophen and Hydroxychloroquine by biosurfactant producing strain *Bacillus velezensis*. Bull. Env. Pharmacol. Life Sci, 12, pp.343-351.

Waghmode, M., and Patil, N.,2023. Isolation, and characterization of *Cladosporium alboflavescens* for Acetaminophen biodegradation. Vidyodaya Journal of Science, 26(02), pp 18-25. https://doi.org/10.31357/vjs.v26i02.6804.

Waghmode, M., Abhyankar, P. and Patil, N., 2024. Mycoremediation of Acetaminophen by the Fungi of Dothideomycetes sp. Isolate. Vidyodaya Journal of Science, 27(01). https://doi.org/10.31357/vjs.v27i01.7497.

*Correspondence: meghmicro2@gmail.com