Analysis of Nitrate Concentrations in River Water Using a Griess Assay

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Laboratory work carried out collaboratively with A. Sitorus

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Nitrate is a nitrogen-containing compound that is essential for plant growth, as it is a vital nutrient used in synthesising proteins and other biological molecules. However, excessive nitrate concentrations in aquatic environments can lead to negative effects such as eutrophication and in turn biodiversity loss (Johnson et al., 2023). One of the main sources of nitrate pollution in the environment is surface runoff from agricultural activity, notably via the use of nitrogenous fertilisers. Overuse of these fertilisers can leach into groundwater systems in excess and subsequently enter rivers and lakes, contributing to elevated nitrate concentrations in freshwater systems (Maier et al., 2009).

High concentrations of nitrate in bodies of water can lead to eutrophication (Lloyd et al., 2019), a process caused by an excess of nutrients. Increased availability of nutrients promotes rapid growth of algae and phytoplankton, resulting in algal blooms. These blooms often reduce the amount of light able to enter the water. This limits the rate of photosynthesis in aquatic plants, causing them to die. Once the algae die, microorganisms decompose them and in turn respire available oxygen in the water. This lowers the concentration of dissolved oxygen and can bring about anoxic conditions that can directly cause the death of aerobic aquatic organisms which reduces biodiversity (Akinnawo, 2023). These dead organisms are then decomposed by a mixture of aerobic and anaerobic saprobionts. The aerobic bacteria respire the remaining dissolved oxygen from the water, and the anaerobic bacteria colonise the water, breaking down all available matter and, in some instances, releasing toxic hydrogen sulfide. This results in the formation of a dead zone, incapable of supporting most aerobic aquatic life.

Additionally, nitrogen-containing compounds can also contribute to environmental acidification. Ammonia released into soil from agricultural sources can undergo nitrification, which produces excess hydrogen ions and subsequently lowers pH, negatively impacting ecosystems. In the UK, a link has been established between higher river nitrate levels and the use of inorganic fertilisers in farming (Whelan et al., 2022). Consequently, the monitoring and management of nitrate levels in river systems near farms are essential for evaluating the impact on the environment. This report investigates the nitrate concentrations in river water samples collected from farms (A–D) and the possible environmental impacts.

Methods

Griess Assay Preparation

River water samples were collected from four farms (A–D). Calibration standards were prepared from a stock solution of $NaNO_3$. 20µl sulphanilamide reagent and 20µl NED reagent were pipetted into 44 wells of a 96-well plate. 100µl of each of the calibration standards was pipetted into 21 of these wells. In 3 other wells, 100µl of ultrapure water was pipetted as a control. Then, 100µl of the river water samples (5 samples taken from each farm A–D) were pipetted into the 20 remaining wells containing the first two reagents.

Absorbance Measurement

The plate was then left in the dark for 10 minutes to allow the reaction to take place. Absorbance was then measured at 540 nm using a plate reader. Using the results from the plate reader, a calibration curve of absorbance against nitrate concentration (µmol) was plotted and used to determine the nitrate concentration of the river water samples.

Results

A calibration curve was produced from nitrate standards using a Griess assay and used to calculate the concentration of the unknown river water samples (Figure 1). Figure 1 shows a positive relationship between nitrate concentration and absorbance at 540 nm.

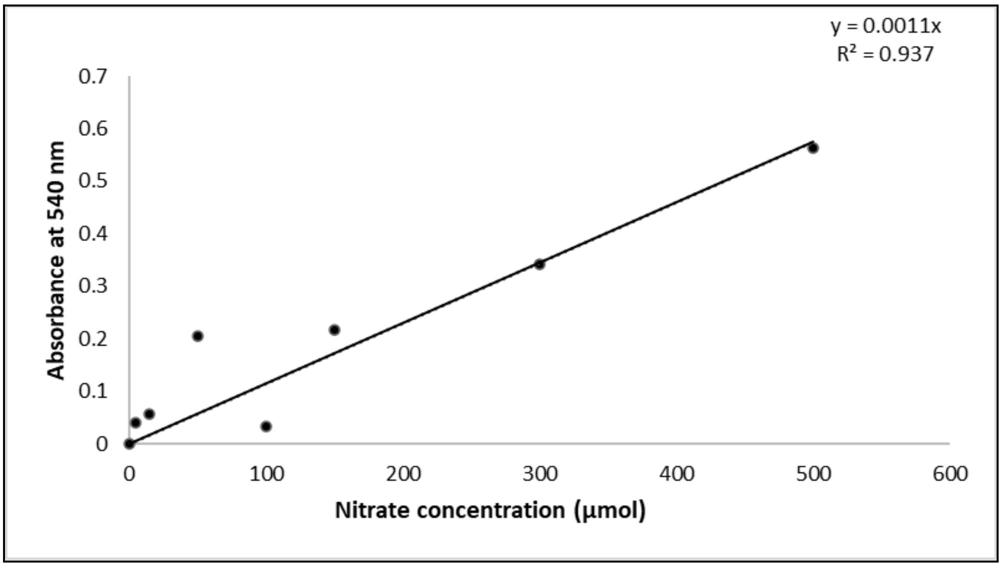

Figure 1. Calibration curve of nitrate standards determined using the Griess assay, showing absorbance at 540 nm plotted against concentration (µmol).

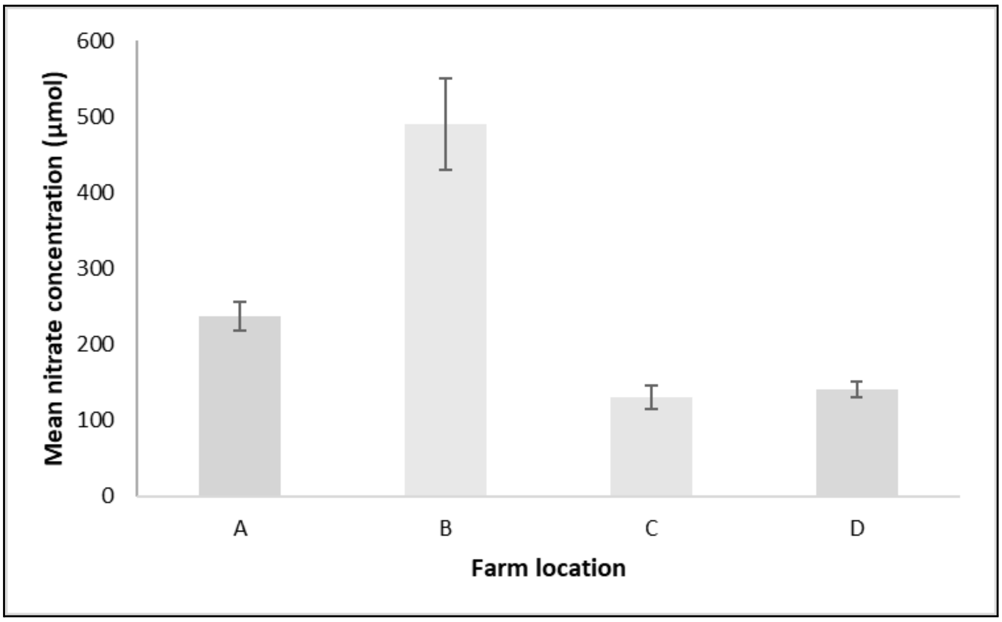

Figure 2. Mean nitrate concentration (µmol) for samples from each farm (A–D) ± standard deviation

Nitrate Concentration

Mean nitrate concentration varied between farms (Figure 2). Farm B (Norton) had the highest mean nitrate concentration, Farm A (Dondini) had intermediate concentrations, while Farms D (Dennis) and C (Kuhnert) had the lowest values.

Variation Between Samples

Variation between samples differed between farms. Farm B had the greatest standard deviation, whereas Farms A, C, and D had relatively low variation.

Discussion

The results show that Farm B (Norton) is likely the source of increased nitrate concentration, considering that it showed the highest mean nitrate levels in comparison to the other farms. This suggests that agricultural practices at this farm could be contributing to the elevated nitrate levels in the river system. One likely source is the increased use of mineral fertilisers, which can drain from the field to the river via runoff (Whelan et al., 2022).

Furthermore, manure and slurry can be carried, particularly in periods of high rainfall, to nearby river systems which can negatively impact water quality (Whelan et al., 2022). During an inspection, the environmental protection officer should inspect the farmer's rate of fertiliser use, the storage and management of manure and slurry, and the proximity of drainage networks to rivers, since all of these factors have the capacity to impact the movement of nitrate from farmland to aquatic environments.

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