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EFFECT OF FLUORINE ON *Rhizobia*: A RESPIRATION-INHIBITION ASSAY

Geretharan T, Jeyakumar P, Bretherton M and Anderson CWN

Fertilizer & Lime Research Centre, Massey University, Palmerston North

Introduction

The continuous (and unintentional) accumulation of fluorine (F) in New Zealand agricultural soils as a result of phosphate fertiliser addition has been well documented (Loganathan et al., 2006). Soil micro-biota are vital to the functioning of the soil ecosystem which underpins on-farm sustainable production (Six et al., 2006), and there is potential risk that elevated soil F concentrations may be harmful to soil microorganisms which are important for nutrient cycling and soil formation. *Rhizobium leguminosarum* is a nitrogen fixing soil bacteria which is a fundamental component in New Zealand legume-based pastoral farming. Any impact of F on *Rhizobium leguminosarum* would have an adverse effect on New Zealand pasture production. Quantifying the effect of F on *Rhizobium leguminosarum* is therefore necessary to ensure that the increasing F concentration of New Zealand soils does not threaten the value of this ecosystem service. Initial efforts are being undertaken to determine Inhibitory Concentration (IC) limits to *Rhizobium leguminosarum* sensitivity to F concentrations as a first step in the development of F guideline values for New Zealand agriculture soils.

Material and Methods

The fluoride IC_{10} value for *Rhizobium leguminosarum* was determined using the MicroResp 96-well format respiration-inhibition assay developed by Campbell et al. (2003). In this context, IC_{10} is defined as the fluoride concentrations that causes 10% inhibition of *Rhizobium leguminosarum* respiration. Three different F salts (NaF, KF and NH_4F) were used in an attempt to differentiate between any cation affect on fluoride ion toxicity. Each well was charged with 0.25 ml of YMbroth (YMB). An equal volume (0.2 ml) of varying concentrations of NaF, KF and NH_4F (0, 0.5, 1, 5, 10, 20, 50, 70, 100, 500 and 1000 mgF^-/L) was added to eight-well columns across the plate. The *Rhizobium leguminosarum* culture was grown in 100 ml of YMB in a shaking incubator (200 rpm) at 27ºC and then inoculated (0.05 ml aliquot) to the 96-well plate which contained F^- and YMB. *Rhizobium leguminosarum* growth in each plate was determined by respiration (amount of CO_2 released from the plate) using 96-well colorimetric gel traps according to the method described by Campbell et al. (2003). An initial absorbance value (A590) was recorded colorimetrically (t=0), then the microplate was sealed with a detection microplate and incubated for 24 hours at 27ºC. After incubation, detection microplate absorbance values were measured (t=24) and absorbance differences (Δ A590) were calculated. Absorbance difference (Δ A590) gives the amount of CO_2 released (Wakelin et al., 2014) from the *Rhizobium leguminosarum* in the presence of F.

Respiration % was calculated by using the following equation.

$$RP(\%) = \left(\frac{\Delta A_{590}}{Mean\ \Delta A_{590}\ of\ control}\right) \times 100$$

A non- linear regression equation was used to determine the IC_{10} value.

Results and Discussion

The effect of fluoride concentrations on *Rhizobium leguminosarum* respiration is summarised in Figure 1.

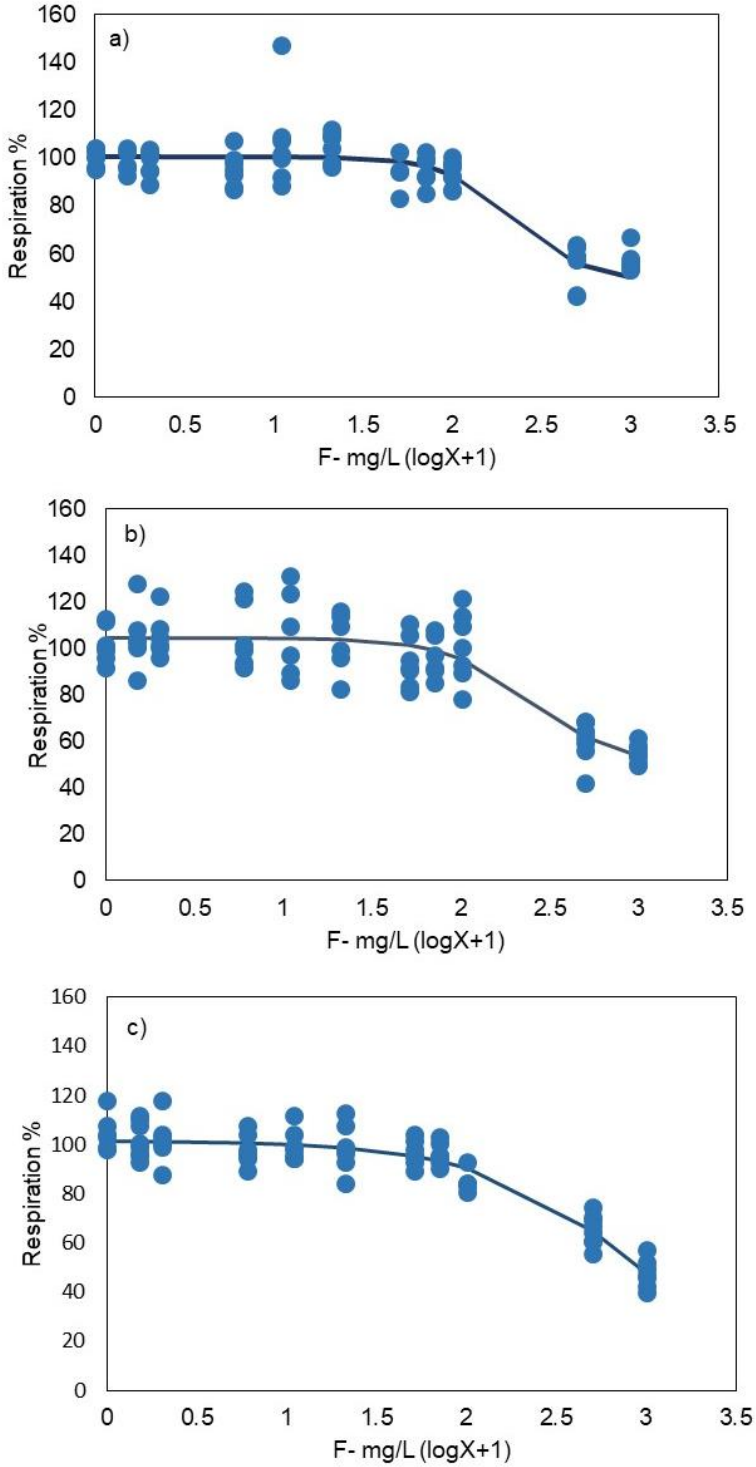

Figure 1. Dose response relationships between fluoride concentrations and *Rhizobium leguminosarum* respiration (% of control). Three different F salts were used (a) NaF (b) KF (c) NH_4F

The IC_{10} values for fluoride toxicity to *Rhizobium leguminosarum* are higher than 100 mgF^-/L for each of the F salts used in the assay (Figure 1). In other words, fluoride inhibits respiration by less than 10% when *Rhizobium leguminosarum* is exposed to fluoride concentration up to 100 mgF^-/L.

When *Rhizobium leguminosarum* was exposed to a fluoride concentration greater than 100 mg/L, respiration was significantly inhibited. Barbier et al. (2010) reported that fluoride ions inhibit the enzymes required to the glycolytic pathway of the Krebs cycle leading to suppression of cell respiration. Cittanova et al. (1996) reported that fluoride changes mitochondria activity which is an important cellular organ for respiration.

Gao et al. (2012) reported that exchangeable and water soluble fluorides are highly available to microorganisms and these are the forms of soil F that are toxic to microorganisms. Loganathan et al. (2006) measured the water soluble F concentration of 27 pasture sites in New Zealand and reported water soluble F concentrations ranging between 0.5-4.8 mg/kg, with a free fluoride concentration in soil solution less than 0.04 mg/L (as defined in this work, soil solution F concentration is determined by multiplying the water extractable F concentration by a factor of 0.09 (Loganathan et al., 2006)). This range of 'real life' soils solution F^- concentrations (<0.04 mg/L) is at least 3 orders of magnitude lower than the IC_{10}value (100 mg/L) determined for 3 different F salts in this study.

Conclusion

This study concluded that *Rhizobium leguminosarum* respiration was inhibited less than 10% for a fluoride concentration up to 100 mg/L. All laboratory toxic effects of F on *Rhizobium leguminosarum* have been recorded at solution F concentrations that are 3 orders of magnitude higher than those recorded for New Zealand agricultural soils under 'normal conditions'. There appears to be no indication of imminent risk of soil F to *Rhizobium leguminosarum.*

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