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# INTEGRATED EFFECT OF DEPLETED URANIUM AND SOIL PROPERTIES ON MAIZE (*ZEA MAYS* L.) GROWTH

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**Abstract**

Depleted Uranium (DU) is primarily composed of the isotope uranium-238 ($^{238}U$). It has been identified as an emerging pollutant with the advancement of nuclear science, especially in the regions where nuclear weapons had been used in the recent past. Effect of DU on maize growth was studied by using 2 soils of contrasting characteristics (cambisol and podzol) in a growth chamber study. Both soils were amended with increasing concentrations (0, 0.5, 1.0, 2.5, 5 and 10 mM) of DU as $UO_2(NO_3)_2$ and $KNO_3$. Three day old maize seedlings were sown under optimum growth conditions and harvested after 2 weeks. Data regarding plant height, SPAD-meter reading and fresh and dry biomass were recorded and analyzed statistically. Effect of different concentrations of DU and $KNO_3$ were also monitored on post harvest soil microbial activity through infra red gas analyzer (IRGA) respiration and substrate-induced respiration. Results revealed that there was no significant effect of DU on maize growth when compared with $KNO_3$ for cambisol soil at all concentrations. However, dry shoot weight of maize in podzol soil decreased significantly at 10mM of DU compared to $KNO_3$ (0.22 vs. 0.36g). Results of IRGA and substrate induced respiration revealed that there was no significant difference among $CO_2$ evolved at various concentrations of DU and $KNO_3$ for both soils. Overall, cambisol soil produced more maize biomass (2 folds) and higher microbial activity (up to 2.8 folds) compared to podzol soil. The study concluded that effect of DU on maize growth was directed by the soil physico-chemical properties and productivity status of 2 soils.

## Introduction

The development of nuclear science and technology has led to environmental contamination caused by radio nuclides, in particular by uranium (Gavrilescu *et al*., 2009). Depleted Uranium (DU); primarily composed of the isotope uranium-238 ($^{238}U$) is the by-product of nuclear industrial process used to enrich natural ore for use in nuclear reactors (Jia *et al*., 2006). DU differs from naturally occurring uranium by virtue of having most of its $^{235}U$ and $^{234}U$ isotopes content removed in the enrichment of fuel reprocessing, hence it possesses 99.79% $^{238}U$ by mass (Radenkovic *et al*., 2008). DU has become an environmental concern in recent era due to its increased use in weapons deployed in different regions of the world such as Bosnia, Afghanistan and Iraq (Jia *et al*., 2006). Soluble forms can migrate to surface and groundwater potentially contaminating drinking water supplies, be taken by plants or aquatic organisms or volatilized (Knox *et al*., 2008). Preserving microbial community activity in soil is also vital for preserving ecosystem functioning (Jalaluddin & Hamid, 2011). However, the short and long term influence of DU on soil microbial populations remains largely understudied.

Plants also possess the potential to take up DU present in soil and associated water bodies (Neves *et al*., 2008). Plants growing in contaminated areas were reported to take up to 100 times more U compared to plants of other areas (Alloway, 1990). Uranium in soil does not often create a radiological hazard to humans, but can cause toxicity to plants (Sheppared *et al*., 1992). The dangers arising from the biochemical toxicity of U as a heavy metal are considered to be about six times higher than those from its radioactivity (Schnug *et al*., 2005). The information on the U phytotoxicity is yet contradictory; levels as low as 5 mg $kg^{-1}$ in soil have been considered as toxic, whereas many studies reported absence of toxicity at U levels 100 to 1,000-fold higher (Sheppard *et al*., 1992).

The use of plants to extract metals and radio-nuclides from contaminated soil and water has been explored as an economical approach (Achakzai *et al*., 2012). Little information is available on the accumulation of U in plants grown in U contaminated soils. It is generally observed that plants also vary greatly in their U uptake capacities (Duquene *et al*., 2006). Moreover, uranium behavior in soils is controlled by actions and interactions between physico-chemical and biological processes that also determine its bioavailability (Laroche *et al*., 2005). The solubility of uranium in soil is dependent on several factors such as: pH, redox potential, temperature, soil texture, organic and inorganic compounds, moisture and microbial activity (Rivas, 2005).

The objective of this work was to study the integrated effect of DU and soil properties on maize growth in growth chamber and their effect on soil microbial activity.

## Materials and Methods

**Soil analysis:** Two soils (eutric cambisol and haplic podzol) with contrasting physic-chemical characteristics were obtained from the store house of School of Environment and Natural Resources, Bangor University. Both soils were air dried, sieved through 2 mm and analyzed for: particle size distribution by sieving and sedimentation after sodium hexametaphosphate distribution; pH in a water suspension (1:2.5 soil/water); electrical conductivity (EC) of the extract saturation; total C and total N on CN analyzer, Ca and K on flam photometer and available uranium after 1M ammonium acetate extraction (Schollenberger & Simon, 1945) on ICP-MS.

**Growth chamber experiment on maize:** Eutric cambisol and haplic podzol soils were weighed out (200 g) into 200 ml polypropylene pots. Five increasing concentrations (0.5, 1, 2.5, 5 and 10 mM) of $KNO_3$ and DU as uranyl nitrate, $UO_2(NO_3)_2$ were made in 100ml volumetric flasks and stored in plastic storage bottles. The soils taken in the pots were amended with 20 ml of increasing concentrations of $KNO_3$ and DU (10% of the soil weight). The control was amended with distilled water. The pots were first emptied and refilled with constant spraying of respective solutions of DU or $KNO_3$ with pasteur pipette to aid uniform dispersal. Thus there were 36 pots for each soil with 12 treatments and three repeats.

Maize hybrid (cv. KSW) seeds were sown in an incubator at 70% relative humidity (RH) and 25°C under darkness. Three day old uniform seedlings were sown with each pot containing three seedlings. Pots were put in the growth chamber in completely randomized design with 16/8 hours light and dark period. Temperature and RH for light and dark period were adjusted at 25°C, 70% and 20°C, 80%, respectively. Soil in the pots was kept up to field capacity by regular monitoring and irrigation of the pots.

**Data collection and statistical analysis:** Maize plants were harvested after two weeks and data for plant height, SPAD-meter reading, fresh shoot weight and dry shoot weight were recorded. The data was statistically analyzed and graphs were plotted. The results at lower and higher values of $KNO_3$ and DU for all parameters of both soils were compared by applying T-test.

**Effect of DU on post harvest soil respiration-Infera red gas analyzer (IRGA) study:** Effect of increasing concentrations of DU and $KNO_3$ on soil respiration in post harvest soil was monitered. Thirty gram root free soil from each pot of maize trial was weiged into 50ml polypropylene vials. There was 12 treatments with three repeats i.e. 36 vials for each soil. In each cycle, 12 vials were connected to 12 channels of IRGA for four hours. The process was repeated in triplicate for each soil and data was recorded and saved in excel automatically in the PC attached with IRGA. The value of $CO_2$ given off was measured in pmol $CO_2$ channel$^{-1}$ second$^{-1}$. The data obtained by IRGA was analyzed statistically.

**Effect of DU on substrate-induced respiration of post harvest soil:** The effect of increasing concentrations of DU and $KNO_3$ on substrate-induced respiration of post harvest soil was also monitered. Five gram soil from each pot of maize trial was weiged into 50ml polypropylene vials. There was 12 treatments with three repeats i.e. 36 vials for each soil. Soil in each vial was amended with 500µl of 10mM glucose solution having 0.96 KBq ml$^{-1}$ radiolabeled $^{14}C$ and mixed gently. Sodium hydroxide (NaOH) traps containing 1ml of 1 M NaOH, were gently lowered onto the soil surface and tubes were sealed by a rubber bung. The traps were removed after 30 minutes. To each NaOH trap, 4 ml scintillation fluid was added and mixed well on vertex mixture. The value of $^{14}CO_2$ given off was measured on liquid scintillation counter (Wallace 1409: Turku, Finland). The data obtained by scintillation counter was statistically analyzed and graphs were plotted.

## Results and Discussion

**Effect of DU on maize growth:** Effect of DU on different growth parameters of maize growth for cambisol soil is presented in Figs. 1a to 4a and for podzol soil is presented in Figs. 1b to 4b. In case of cambisol soil, data of plant height showed non significant difference against different concentrations (0.5 to 10 mM) of $KNO_3$ and DU ranging from 28.1 to 30.5 cm. Similar results were also obtained in case of SPAD-meter reading, 21 at control and 26 at 10 mM of $KNO_3$, statistically non-significant.

Results of fresh shoot weight revealed that both $KNO_3$ and DU produced 4.4 g shoot weight at 10 mM, they were was no-significant with 3.9 g produced at control. Almost similar results were achieved regarding dry shoot weight for cambisol soil i.e., 0.65g at control, and 0.74 and 0.76g at 10 mM for DU and $KNO_3$, respectively.

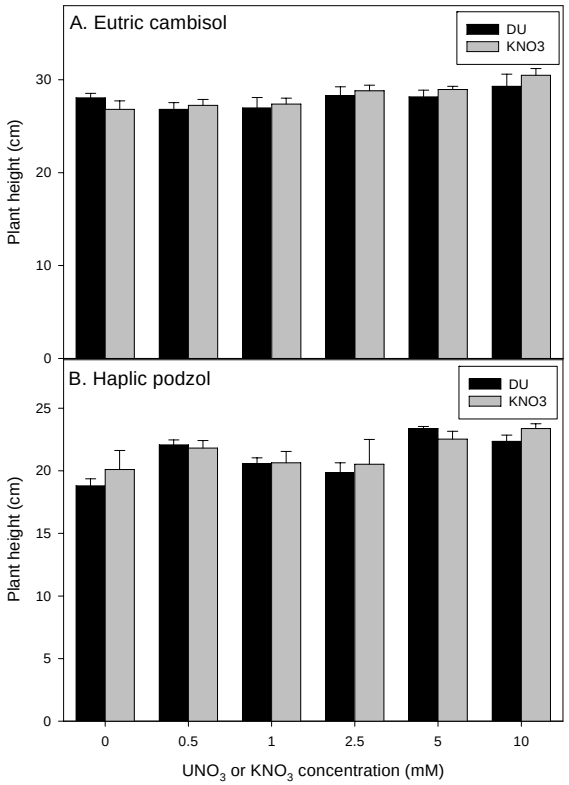


Fig. 1. Effect of different concentrations of $UNO_3$ or $KNO_3$ on plant height of maize.

Regarding podzol soil, data of plant height showed non significant difference against different concentrations (0.5 to 10 mM) of $KNO_3$ and DU ranging from 18.8 to 23.4 cm. Similar results were also obtained in case of SPAD-meter reading, 22 at control and 27 at 10 mM of $KNO_3$, statistically non-significant. In contrast to cambisol soil, results of fresh shoot weight of maize in podzol soil revealed that at 10 mM $KNO_3$ shoot weight produced 1.9g was significantly higher than 10 mM DU i.e., 1.5g (Table 2). Almost similar results were achieved regarding dry shoot weight for podzol soil i.e., 0.36g at 10 mM of $KNO_3$ which was significant higher than 0.22g at 10 mM of DU (Table 2).

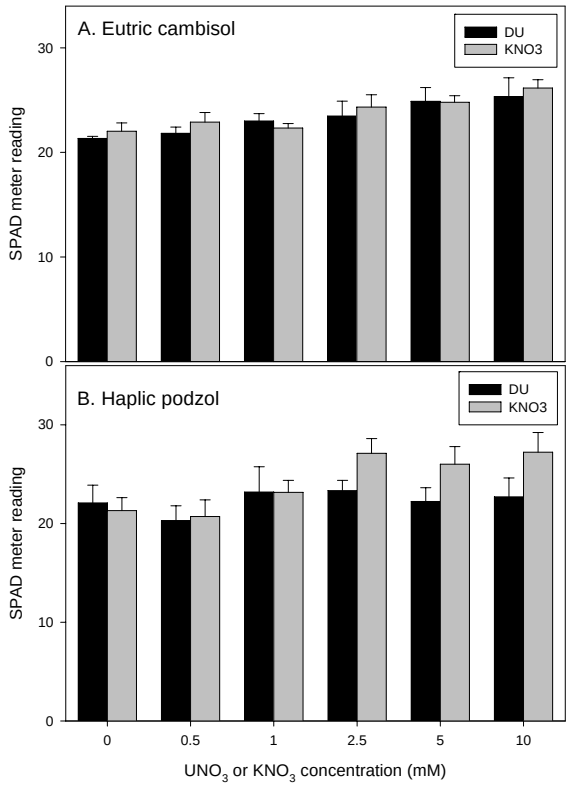


Fig. 2. Effect of different concentrations of $UNO_3$ or $KNO_3$ on maize SPAD-meter reading.

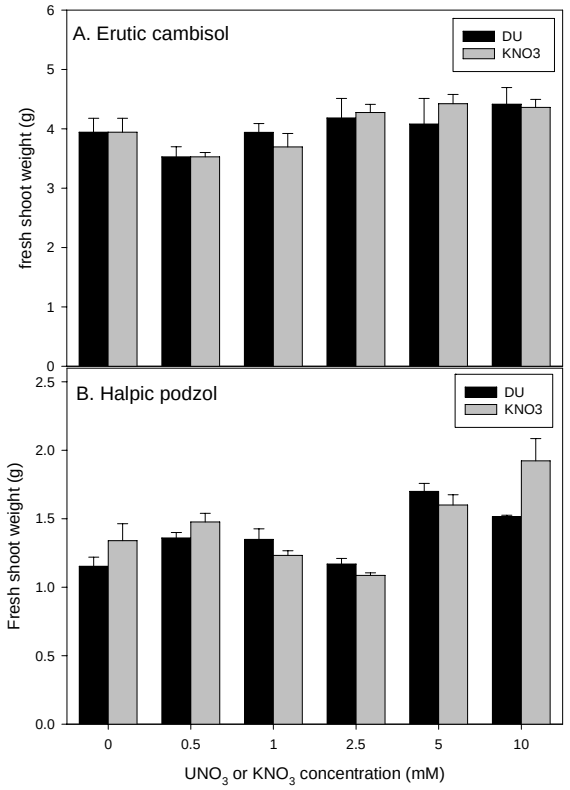


Fig. 3. Effect of different concentrations of $UNO_3$ or $KNO_3$ on maize fresh shoot weight.

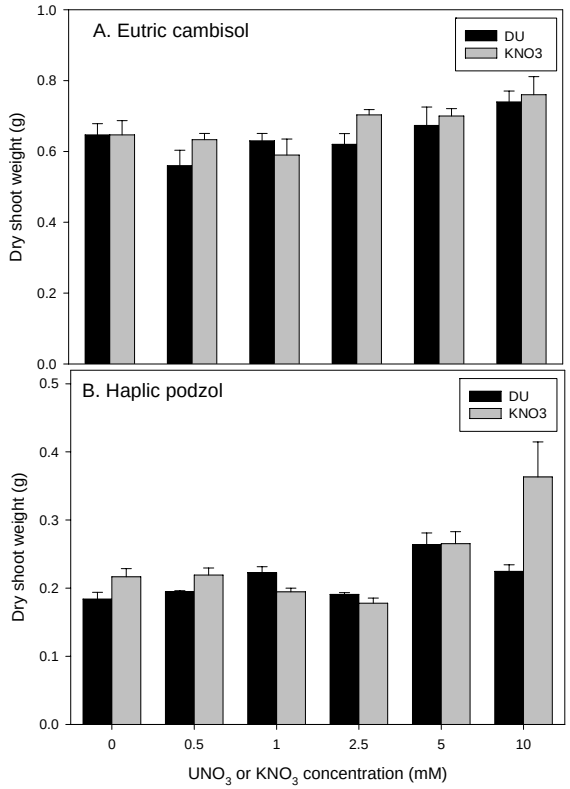


Fig. 4. Effect of different concentrations of $UNO_3$ or $KNO_3$ on maize dry shoot weight.

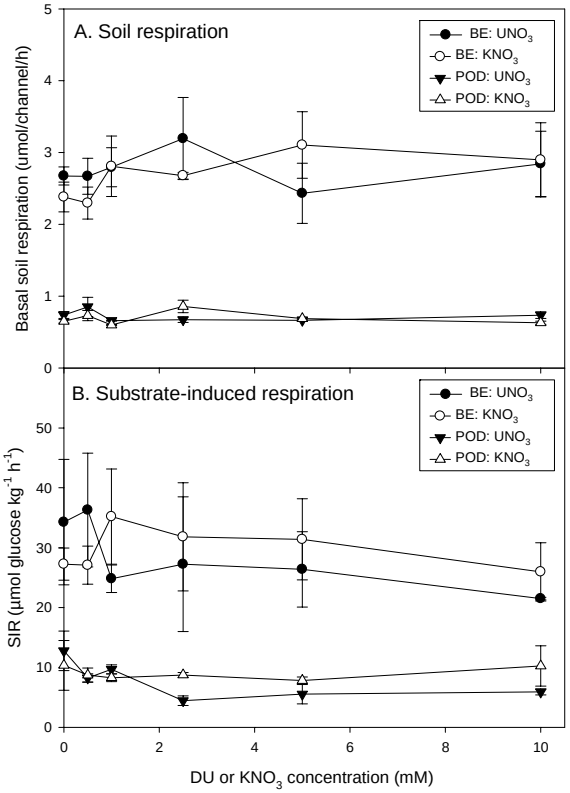


Fig. 5. Effect of different concentrations of $UNO_3$ or $KNO_3$ on soil respiration.

Results of dry shoot weight of maize showed non significant difference in the cambisol soil (Fig. 3a) at all concentrations of DU and $KNO_3$. But in the podzol soil it differed significantly with each other (0.22 vs. 0.36 g) at 10 mM concentration (Fig. 3b). SPAD-meter reading showed an ascending trend with increasing concentrations of DU and $KNO_3$ ranging from 20 to 27 (Fig. 4a) in cambisol soil. In podzol soil the response was also more or less the same (Fig. 4b). However, statistically there was no difference between similar concentrations of DU and $KNO_3$ when compared with each other in both soils.

Comparison of cambisol and podzol soil revealed that dry biomass production of cambisol soil was highly significant than podzol. This premise is also supported by Figs. 6a & 6b and Table 1 as uranium behavior is controlled by physio-chemical characteristics of soils and its bioavailability to plants (Laroche *et al*., 2005). The least amount of U was found in exchangeable and soluble form (Shahandeh & Hossner, 2002). Due to low pH and less organic matter the bioavailability of DU might be increased in podzol soil as compared to cambisol soil.

**Effect of DU on substrate-induced respiration of post harvest soil:** Results of substrate induced respiration are given in Fig. 5a. It is revealed from the graph that there was non-significant difference among various concentrations (0.5 to 10 mM) of DU and $KNO_3$ for both cambisol and podzol soils regarding µmol $CO_2$ channel$^{-1}$ h$^{-1}$ evolution. The graph also shows that overall there was more microbial activity in the Eutric cambisol (2.8 folds at control) at all concentrations of DU or $KNO_3$ compared to the podzol soil. It might be due to poor nutrient and organic carbon status of podzol in contrast to cambisol (Table 1).

**Table 1. Physic-chemical characteristics of eutric cambisol and haplic podzol soils.**

| Parameter | Unit | Eutric cambisol | Haplic podzol |
|---|---|---|---|
| Texture | - | Sandy clay loam | Sandy loam |
| pH | - | 6.43 | 5.22 |
| EC | dSm$^{-1}$ | 0.02 | 0.05 |
| Water Holding cpacity | % | 32.4 | 22.6 |
| Total carbon (TC) | mg l$^{-1}$ | 18 | 11 |
| Total Nitrogen (TN) | mg l$^{-1}$ | 27.9 | 17.4 |
| Potassium (K) | mg l$^{-1}$ | 0.95 | 0.48 |
| Calcium (Ca) | mg l$^{-1}$ | 6.81 | 0.29 |
| Uranium (U) | mg l$^{-1}$ | ND* | ND |

*****Not detectable

**Table 2. Comparison of physiological growth parameters of maize for cambisol and podzol soil.**

| | Plant height (cm) | | SPAD-meter reading | | Fresh shoot weight (g) | | Dry shoot weight (g) | |
|---|---|---|---|---|---|---|---|---|
| | Cambisol | Podzol | Cambisol | Podzol | Cambisol | Podzol | Cambisol | Podzol |
| DU | 29.3 ± 0.6 | 22.4 ± 0.8 | 25 ± 0.8 | 23 ± 0.8 | 4.4 ± 0.6 | 1.5 ± 0.8 | 0.74 ± 0.8 | 0.22 ± 0.8 |
| $KNO_3$ | 30.5 ± 0.3 | 23.4 ± 0.4 | 26 ± 0.4 | 27 ± 0.4 | 4.4 ± 0.3 | 1.9 ± 0.4 | 0.76 ± 0.4 | 0.36 ± 0.4 |
| T-test | 0.13 | 0.13 | 0.84 | 0.85 | 0.13 | 0.02 | 0.82 | 0.02 |

**Effect of DU on post harvest soil respiration-Infera red gas analyzer (IRGA) study:** Results of substrate induced respiration are given in Fig. 5b. It is revealed from the graph that there was no significant difference amongst the various concentrations (0.5 to 10 mM) of DU and $KNO_3$ for both the cambisol and podzol soils regarding µmol glucose kg$^{-1}$ h$^{-1}$ consumption. The graph also showed that overall there was more microbial activity in the cambisol (1.6 folds at control) at all concentrations of DU or $KNO_3$ compared to the podzol soil. It might be due to poor nutrient and organic carbon status of podzol in contrast to cambisol (Table 1).

The soil and microbes interaction is very important for plant growth and bioremediation of metal contaminated soils. Plants also stimulate the soil microorganisms through the release of nutrients and transport of oxygen to the roots (Gavrilescu *et al*., 2009). Soil acidity/alkanity and nutrient availability (such as nitrates, phosphates, carbon source and minerals) affect the microbe and plant activities. Under appropriate conditions, microorganisms can affect the stability and mobility of U in soil by altering the chemical speciation, solubility and sorption properties and thus could increase or decrease the concentrations of U in solution and the bioavailability (Gavrilescu *et al*., 2009).

## Conclusion

Results of the study concluded that soil physico-chemical characteristics and uranium interaction affects the bioavailability of uranium, hence it resulted differently on maize plant growth under different soils. The study also implies that soils with higher organic matter showed more microbial activity than that less OM. Further investigations with higher concentrations of U on microbial activity and subsequently its bioavailability under different soil physic-chemical properties will be helpful to study its effects on growth of variety of plants.

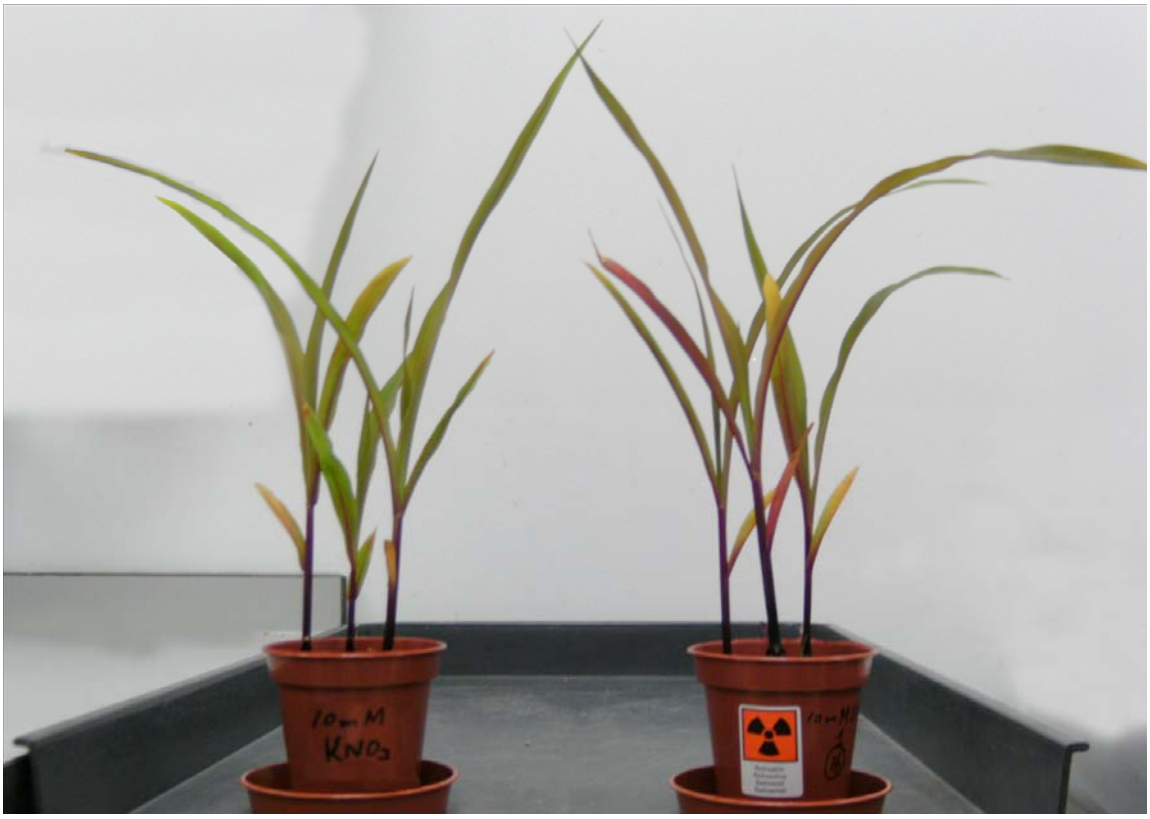


Fig. 6a. Effect of (10 mM) DU and $KNO_3$ on maize growth (Cambisol soil).

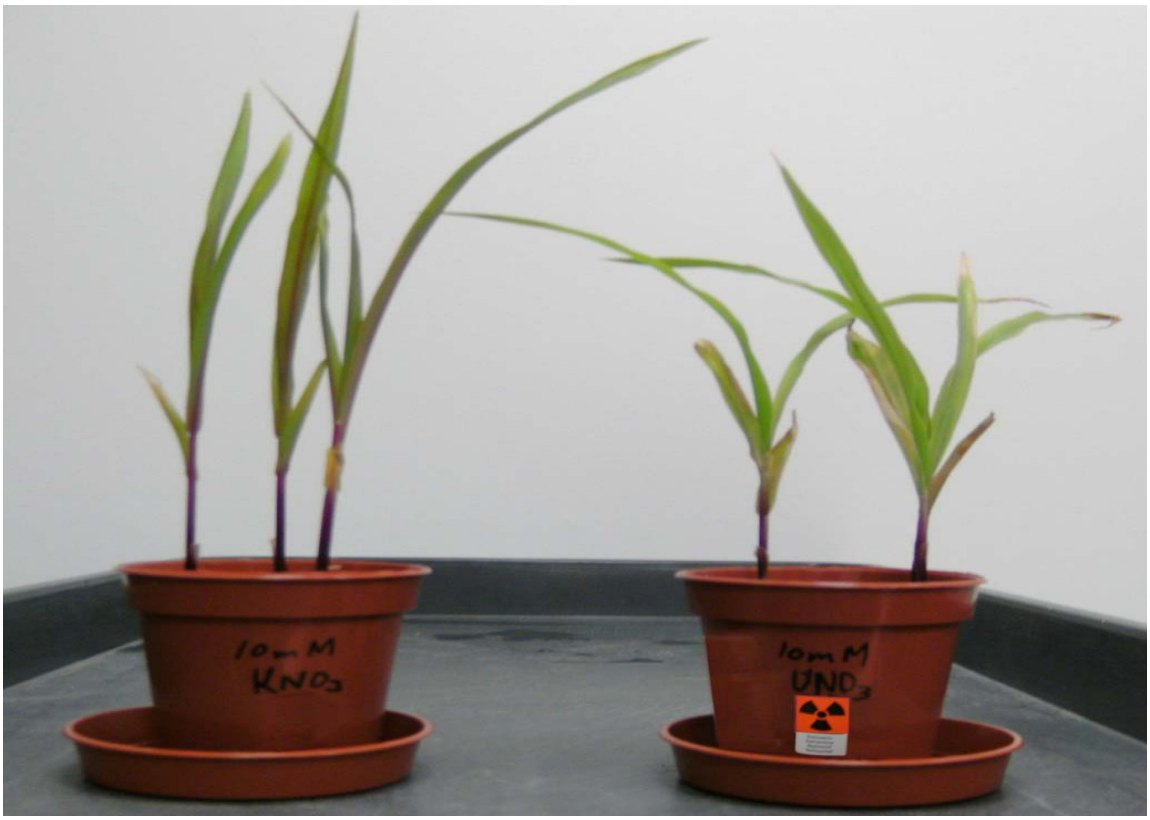


Fig. 6b. Effect of (10 mM) DU and $KNO_3$ on maize growth (Podzol soil).

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