Grasses and Ruminants That Will Help Save Space Ship Earth

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Grasses and Ruminants That Will Help Save Space Ship Earth

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Abstract

For the last twenty years, it has been known that grasses are capable of extracting toxins from the soil. More recently, it has been shown that microorganisms from ruminants, especially sheep, can biodegrade certain toxins in plants and soil, including munition residues. The combination of these two processes act as an agricultural means to clear toxins and munitions from land has been termed **Phyto-Ruminal-Bioremediation** by the United States Department of Agriculture (USDA) as illustrated in the discussion below. As an example, plants containing toxins such as pyrrolizidine alkaloids can be cleared from pastures using sheep and their ruminant microorganisms. Use of grasslands and certain grasses is also being used to clean up other toxins such as munitions in areas where residues of explosives have been left following wars, especially in the middle east. This includes Kuwait's Desert Storm, as well as, Egypt's battles in World War II. It has been documented that when a bomb explodes or a cannon fires, 15% of the munitions is non-oxidized and lies as a toxic residue on the soil. This is true even after many years pass by, ie. nearly 80 years. The use of the Phyto-Ruminal-Bioremediation technology has the ability to revitalize "war-torn" areas into sustainable pastures for animal production and food production for human populations.

The presentation will establish the scientific basis for this new agricultural based technology, **Phyto-Ruminal-Bioremediation.** This scientific approach to clean-up pollutants is a new paradigm for bioremediation. Grasses and ruminates have the potential to make this world a better place.

Introduction

The impetus for this new paradigm had simple beginnings. In veterinary medicine, it was known that sheep were resistant to the pyrrolizidine alkaloids (PAs) that are contained in certain toxic weeds such as tansy ragwort (*Senecio jacobaea*). With these toxins, while sheep were resistant, cattle and horses were not. When cattle and horses repeatedly ate this common weed in the pastures, they developed and died from a chronic liver disease. This left the question of why were sheep resistant and cattle and horses not, especially cattle who are also ruminants like the sheep. Initial thoughts by some scientists were that the liver in sheep was able to detoxify the PAs while the liver in cattle could not. Our lab discovered that the ruminal microbes of sheep were different and able to detoxify the PAs before the alkaloids went systemic in the animal. (Wachenheim et al. 1992; Craig et al. 1992). So a series of experiments at Oregon State University and USDA were conducted *in-vitro* and *in-vivo* with sheep and cattle. The experiments were conducted to determine the degree of protection from the ruminal microbes when the animals were either (1) fed plant material or (2) when extracted PAs were infused into the liver (Hovermale et al. 2002: Duringer et al. 2004). These experiments were able to determine that the sheep ruminal organisms were the protective entity (Ivey et al. 2005).

These conclusions led us to an insight that possibly ruminal microbes could degrade other nitrogenous compounds such as found in munitions. Moveover, plant material that existed 65 million years ago is now oil. Could both of these substances and their molecules be degraded by ruminal microbes? This paper will concentrate on munitions. This led to a series of three types of experiments to determine: first, could plant material bring up munitions from the contaminated soil; second, could sheep ruminal microbes either *in-vitro* or *in-vivo*, biodegrade these nitrogen containing munitions into non-toxic molecules and, finally, could sheep be fed radiolabeled TNT (2,4,6,trinitrotoluene) and break it down into non-toxic moieties with no harm to the sheep (Smith et al. 2008).

The following experiments were conducted primarily at the Endophyte Testing Laboratory at OSU in Corvallis Oregon and USDA to develop "proof of concept".

Methods and Results.

Experiments 1. *Determination that grasses could incorporate munitions and munitions residues in vitro using radiolabeled TNT.*

A study was undertaken to determine if three types of grass could bring up and incorporate radiolabeled TNT in their leaves. The grass species were tall fescue (*Festuca arundinacea, "SR4600"),* perennial ryegrass (*Lolium perenne*, "Century") and orchard grass (*Dactylis glomerate*). Chehalis Silt loam was placed in 6 inch pots. The pots had C^{14} TNT added to the soil at 2.8 uCi/mg and 10 mg/ml cold TNT was dripped into the soil. Four pots of each species were seeded with the individual grass species and placed in a greenhouse to grow. Clippings of the grasses were taken at 63, 181 and 369 days after planting. Figure 1a. Autoradiography of the dried leaves were taken plus using HPLC analyses to measure the TNT content. Figure 1b. All three cool season grasses were able to extract TNT from the soil with variations between grasses. This showed that grasses can effectively extract TNT from the soil (Duringer et al. 2010).

Figure 1a. Grasses grow with thick root mass down to 1 meter depth.

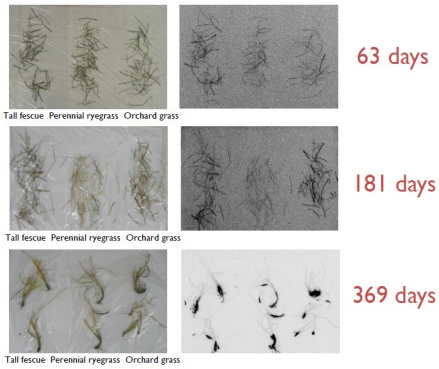

Figure 1b. Left side is a photo of the grasses, Right side is autoradiography of same grass showing radiolabeled TNT content.

Experiments 2. *In-vitro use of ruminal microbes to detoxify TNT, RDX,HMX,*

TNT (2,4,6,trinitrotoluene). The initial study was an *in-vitro* investigating the ability of ruminal microbes from sheep to biodegrade TNT (Fleischmann et al. 2004). Using an artificial rumen, sheep ruminal microbes were collected and incubated with TNT. Figure 2 shows that the initial TNT spike was totally degraded by one hour and only TNT metabolites were remaining. By four hours of incubation, the TNT metabolites were degraded into non-toxic moieties. Sterilized rumen fluid did not result in this TNT metabolism (Fleischmann et al. 2004: Craig et al. 2006; Perumbakkam et al. 2011)

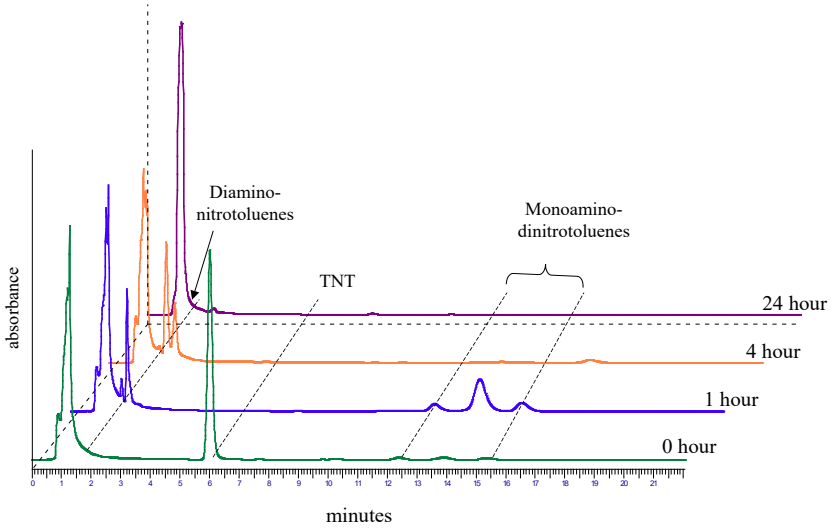

Figure 2. Graph of the time line of ruminal microbes degrading TNT under anaerobic conditions. Pasteurization of these microbes do not result in TNT degradation

RDX (Hexahydro-1,3,,5-trinitro-1,3,5-triazine). A series of in-vitro experiments with whole rumen fluid anaerobically incubated with RDX were conducted. In addition, 16s RNA based genomic extractions were analyzed to identify the responsible bacteria as well as their metabolism (Eaton et al. 2011; 2012; Perumbakkam et al. 2012). Next, genes were identified that degraded the RDX incubated with the sheep

ruminal microbes (Gioriozzo et al. 2013) It should be noted that soil bacteria take several months to years to degrade RDX as well as TNT, whereas ruminal microbes degrade these compounds in hours (Li et al. 2014).

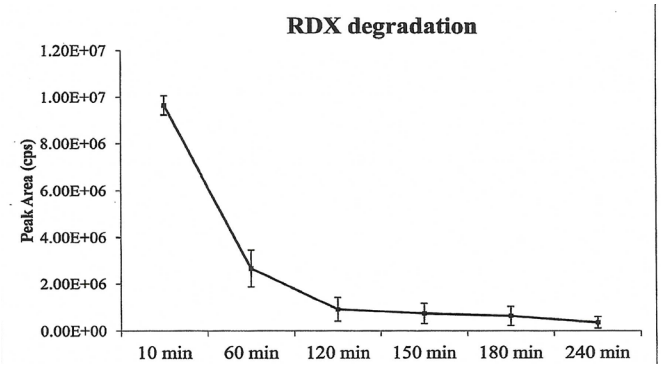

Figure 3. RDX degrades when incubated with ruminal microbes under anaerobic conditions in 2 hours. Not shown is soil microbe degradation which takes years to break apart this molecule (Li et al.; 2014; Ryott et al. 2011)

HMX (Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocane). Correspondingly, the third high explosive nitroamine was investigated for rumen fluid degradation. HMX was incubated with whole rumen fluid similar to the experiments above with TNT and RDX. Pasturized rumen fluid did not degrade HMX whereas live ruminal microbes anaerobically degraded HMX to non-toxic metabolites and small molecules within hours. Moreover, quantitative sequencing for the classification of 16s rRNA of HMX clones was done. (Perumbakkam et al. 2012; Eaton et al. 2014)

Experiment 3. *Feeding sheep with radiolabeled TNT to in-vivo determine the breakdown products by the sheep ruminal microbes. The distribution of the radiolabel, and the health of the sheep during this process.*

Sheep were dosed with 35.5 mg dietary non-labelled TNT for 21 consecutive days. This was an appropriate amount of TNT that would be in plants grown on TNT from contaminated soil. On day 22, a total of 1689 uCi ^{14}C TNT was then administered to the sheep in an oral bolus. The purpose of these studies was to determine the mass balance of the radiolabeled toluene molecules. Extensive extraction protocols were used to measure the TNT, toluene, and the radiolabeled $^{14.}$C TNT. There was a complete mass balance calculated and none of the toluene molecules were present. The radiolabel was only found in short chain carbon molecules. In conclusion, approximate 10% of the carbon molecules were found in the body tissues, 17% was in the urine, and 73% in the feces. It must be noted that no clinical signs were ever observed in the sheep with this treatment (Smith et al. 2008).

Discussion

These studies supports an *agricultural solution* to munition remediation. It is documented that munitions, ie bombs or cannons, when they explode, leave a 10% to 15% residue on the soil. This residue is toxic to animals and humans. These studies demonstrate that cool season grasses are able to absorb and extract TNT from this contaminated soil. Ruminal microbes from grazing sheep complete the reduction as well as break the covalent binding of TNT in the explosive-residue-laden plant material. Ultimately, this results in uncontaminated soil suitable for producing safe feed for animals as well as safe feed for humans.

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