Swedish University of Agricultural Sciences
Department of Soil and Environment

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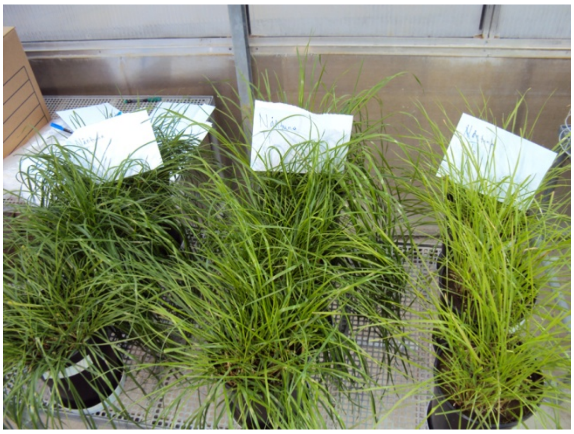

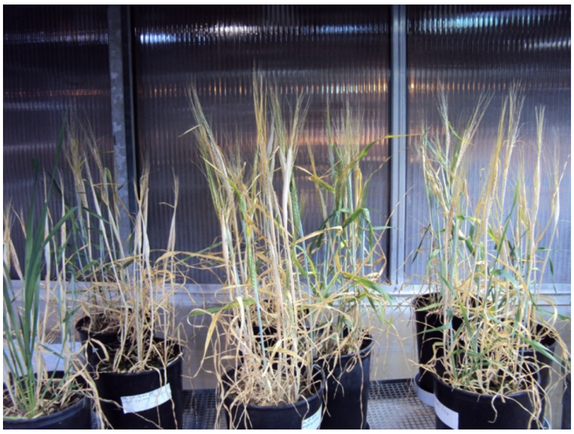

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Cover: Italian ryegrass and spring barley without and with sludge addition (0, 15 and 30 ton/ha respectively), photo by author.

Abstract

Aluminium sulphate ($Al_2(SO_4)_3$) is usually added during the drinking water treatment process in order to precipitate organic and inorganic material, resulting in aluminium-based drinking water treatment residual sludge (WTR). Since 2003 Swedish law no longer permits WTR to be deposited in lakes, and there is an interest to explore the possibility to utilize WTR as soil amendment. The aim of this study was to examine the effects of WTR application on crop growth and concentrations of macronutrients and trace elements (including micronutrients and potential toxic elements). A greenhouse pot experiment was conducted with three soils; a clay loam rich in phosphorous (P), a loamy sand rich in P, and a silty loam with a low soil P concentration, and two crop species Italian ryegrass (*Lolium multiflorum* Lam. cv. Fredrik) and spring barley (*Hordeum vulgare* L., cv. Barbro) mainly used as livestock feed. Addition of WTR at a rate of 30 tons (t) ha^{-1} revealed significantly higher yields (6.3 t ha^{-1}) of Italian ryegrass harvested twice, compared with a control without WTR addition (4.1 t ha^{-1}) for a clay loam. The corresponding value for a loamy sand was 7.0 t ha^{-1} with WTR compared with 4.1 t ha^{-1} for the control. For the third soil studied, a silty loam, yield was only marginally higher with WTR addition. Significantly higher yields were also found after application of 15 t ha^{-1} WRT to these soils. In contrast, spring barley showed no significant increase in yield after WTR application on any of the three soil types. Concentrations of copper (Cu) were significantly higher in Italian ryegrass grown with WTR application (mean 9.3 mg kg^{-1}) compared with the control (7.2 mg kg^{-1}). Both Italian ryegrass and spring barley grown on a clay loam took up a significant amount of sulphur (S) from the WTR-amended soils compared with the same soil with no amendment. On a clay loam with high soil concentrations of Cu (25 mg kg^{-1}), Cu offtake with Italian ryegrass was 0.049 kg ha^{-1}. Molybdenum (Mo), and zinc (Zn) may have been limiting for the growth of Italian ryegrass and spring barley, since their concentrations in plants were significantly higher (28 mg kg^{-1} Mo and 32 mg kg^{-1} Zn) without WTR application than at the higher load (30 ton ha^{-1}) of WTR applied (9 mg kg^{-1} Mo and 30 mg kg^{-1} Zn). Concentrations of the trace elements Ni, Mo, Cu, Zn were below the limit considered toxic to ruminants. However, the latter has only been poorly investigated. Drinking water treatment residuals could be applied to soils like the clay loam and loamy sand studied here as soil amendments when growing crops such as Italian ryegrass and spring barley. For the silty loam, no positive effects or negative effects were apparent.

Key words: WTR, sludge, barley, ryegrass, soil type, trace elements, yield.

Svensk sammanfattning

Upptag av makro- och mikronäringsämnen efter tillsats av vattenverksslam vid odling för foderproduktion

Bodil Lindström och Barbro Ulén

Introduktion

Möjligheterna att sprida vattenverksslam på olika sorters jordar under svenska förhållanden har bara testas i begränsad omfattning (Persson, 1994). Det framkom då att slammet binder lättillgängligt fosfor i marken (P) och har en pH ökande effekt, men att detta inte inverkar på skörden av grödan (vete) eller dess P-upptag. Vattenslammets låga innehåll av tungmetaller och näringsämnen, jämfört med avloppsslam, medför att det inte sker någon tillförsel av tungmetaller om slammet sprids på åkrar, men det ger heller inte någon gödslingseffekt på grödan. En typ av problemjordar som därför kan ha nytta av slamspridning är leriga jordar med högt fosforinnehåll, eftersom de är erosionsbenägna och därmed för bort fosfor från åkrarna till vattendrag och ger upphov till övergödning.
Tillväxt hos grödor styrs av tillgång på vatten, solljus och näringsämnen. De ämnen som behövs i störst mängder är kväve (N), fosfor (P) och kalium (K) men en brist av något av de andra makroämnena svavel (S), magnesium (Mg), kalcium (Ca) eller mikroämnena bor (B), koppar (Cu), järn (Fe), mangan (Mn), molybden (Mo), nickel (Ni) eller zink (Zn) ger likaså allvarliga begränsningar i tillväxten.

Syfte

Som ett led i att testa om vattenverksslam kan spridas på lerrika och fosforrika åkrar i Sverige genomfördes ett odlingsförsök i växthus. Med de optimala odlingsbetingelser som ett växthusförsök erbjuder kan slammets påverkan på grödan mätas enkelt och precist på flera jordar samtidigt, eftersom de platsspecifika väderförhållandenas inverkan på grödan elimineras.
Syftet med detta experiment var att undersöka vilken effekt vattenverksslammet har på ett par vanligt förekommande svenska grödor som används för djurfoder odlade på tre olika jordtyper. De faktorer som undersöktes var grödornas avkastning samt deras innehåll av makro- och mikronäringsämnen.

Metod

Till försöket valdes tre jordar utifrån fosforklass och jordart där Krusenberg (Kr) representerar en mellanlera (38% ler) med högt fosforinnehåll, tillskillnad från Säby (Sä) som är en lättlera (20% ler) med måttligt fosforinnehåll. Som kontrast till de leriga jordarna testades en sandig jord med högt fosforinnehåll, Nåntuna (Nå). Tabell 2 visar fördelningen av jordarnas kornstorlekar och tabell 3 ger en översikt av jordarnas och slammets förråd av tillgängliga makronäringsämnen vid försökets början. Dessutom analyserades jordarna och vattenslammet på totalkoncentrationer av makro- och mikronäringsämnen (tabell 4 och 5). Samtliga jordar inhämtades från Uppsalatrakten och togs från 5-20 cm djup och motsvarar således matjorden. I dessa jordar såddes sädesslaget vårkorn (*Hordeum vulgare* L., sorten Barbro) respektive vallgräset italienskt rajgräs (*Lolium multiflorum* Lam., sorten Fredrik). Dessa representerar grödor som används som djurfoder i Mellansverige.

Till dessa tre jordar med vårkorn eller italienskt rajgräs tillfördes två måttliga nivåer av slam motsvarande 15 respektive 30 ton slam per hektar av samma storleksordning som man brukar tillföra stallgödsel. Utöver dessa behandlingar upprättades kontrolled utan slamtillförsel för varje jord och växtart. Varje försöksled upprepades tre gånger. I varje kärl (5 L, 0,0314 m^2) odlades 15 plantor av rajgräs respektive 7 plantor vårkorn. Tätheten valdes utifrån uppskattat behov av utrymme för att plantorna inte skulle vara begränsade av inomartskonkurrens. Växterna odlades under optimala betingelser i växthus (20 timmar ljus 20°C dag/15°C natt) och bevattnades med avhärdat vatten för att inte tillföra några mikronäringsämnen via bevattningen. Totalt gödslades försöket med kväve (N), fosfor (P) och kalium (K) motsvarande 220:66:220 kg N:P:K per hektar. Gödslingen skedde vid fyra tillfällen, dels vi försökets början, dels efter att rajgräset skördats en första gång och sedan ytterligare två gånger då kontrollerna av italienskt rajgräs på Nå-jorden och Kr-jorden uppvisade tecken på näringsbrist, dvs blev ljust gröna (se bilder på framsidan). Alla led gödslades med lika mycket och vid samma tillfällen för att möjliggöra jämförelserna av slamtillförseln, men detta betyder att grödorna gödslades med mer N:P:K än vad som sker i vanliga fall i fält. Detta är vanligt vid odling i kärl eftersom jordvolymen är så pass begränsad för växterna jämfört med i fält.

Under de 13 veckor försöket pågick skördades det snabbväxande italienska rajgräset två gånger medan vårkornet en gång, vid begynnande mognad. Proverna torkades vid 55°C i två dygn varefter de vägdes och sedan maldes i en titan-kvarn, som tidigare kontrollerats för att inte kontaminera proverna med ämnen som vi vill undersöka. Växtproverna analyserades på makro- och mikro-näringsämnena Ca, P, K, Mg, Na, S, Al, Cu, Fe, Mn, Mo, Ni och Zn. Vid försökets slut togs jordprover från alla kärl där Italienskt rajgräs vuxit och analyserades på pH. Jordproven slogs sedan ihop till ett generalprov för varje led från respektive jord varefter generalproven analyserades på den växttillgängliga poolen av ämnena P, K, Mg, Ca, Al, Fe och syraextraherbara poolen av P, K och Cu.

Resultat och diskussion

Skörd

Tillförsel av vattenslam ökade avkastning av både första och andra skörden av det snabbväxande Italienskt rajgräset på mellanleran Kr och sandjorden Nå som hade högt P innehåll (tabell 6). Första skörden av Italiensk rajgräs odlat på lättleran Sä med måttligt P innehåll fick lägre avkastning till följd av tillförseln av vattenslam medan andra skörden tillsynes var opåverkad av tillförseln. Jämför man avkastningen av Italienskt rajgräs på de tre jordarna ser man att kontrollerna av Kr och Nå ligger lägre än Sä's kontroll, som i sin tur hade liknande avkastning som behandlingarna med vattenslam på Kr och Nå. Skördeökningen på Kr och Nå orsakade av vattenslammet gav alltså en avkastning som är i nivå med Sä utan vattenslam. Dessutom var det just kontrollerna av Italiensk rajgräs på Nå och Kr som uppvisade näringsbristsymptom och som föranledde extra gödsling av NPK på alla behandlingar och jordar. Överlag var andra skörden större än den första. Avkastningen av vårkorn påverkades inte signifikant av tillförsel av vattenslam på någon av jordarna. Det kan dock noteras att skördemedelvärdena och de relativt låga sannolikvärdena för Kr (p=0,0965) och Nå (p=0,1014) indikerar att vattenslammet kan haft en skördeökande effekt även på vårkornet.

Koncentrationer av makro- och mikronäringsämnen i skördat material

Makro- och mikroanalyser av växtmaterialet från första skörden av italienskt rajgräs visar att koncentrationerna av de flesta ämnena inte påverkades nämnvärt av vattenslamstillförseln (tabell 7a och appendix 1). Det ämne som främst påverkades var koncentrationen av S, som fördubblades på grund av vattenslamstillförseln på Kr och Nå. Även på Sä fanns det tendenser till ökade S koncentrationerna (p=0,0788) men här låg S-nivåerna på kontrollerna redan i nivå med de som uppnås på Kr och Nå med vattenslamtillförseln. Molybdenkoncentrationen minskade och Cu koncentrationen ökade tydligt medan K visade tendenser på att öka (p=0,0524) på Kr, men dessa ämnen var tillsynes opåverkade på Nå och Sä.
Andra skörden av italienskt rajgräs, som skedde samtidigt som den enda skörden av vårkornet och 13 veckor efter sådd, visar att upptaget av flera ämnen påverkats av vattenslamtillförseln jämfört med första skörden (tabell 7b, appendix 2). Återigen hade S koncentrationerna ökat med tillförseln och denna gång tydligt på alla tre jordarna, däremot var S koncentrationerna generellt lägre jämfört med första skörden, detta trots att andra skörden var större än den första. Kaliumkoncentrationerna i andra skörden italienskt rajgräs på Kr och Nå ökade, medan Mg koncentrationerna minskade, på grund av vattenslamstillförseln. På Kr ökade Ca koncentrationerna och Mo minskade medan Zn koncentrationerna var lägre på de behandlade leden jämfört med kontrollen på Nå-jorden.
Koncentrationerna av makro- och mikronäringsämnena i vårkorn i påverkades inte i lika hög grad av vattenslamtillförseln som italienska rajgräset. Det fanns dock ett par likheter mellan arterna vad gäller vilka ämnen som påverkades. På Nå-jorden ökade S-koncentrationerna och Zn koncentrationerna var lägre på de behandlade leden jämfört med kontrollen på grund av vatten-slamstillförseln (tabell 7c, appendix 3). Vårkornet avvek från italienskt rajgräs genom att P-koncentrationen påverkades av vattenslamstillförseln och minskade, detta skedde också på Nå-jorden.
Koncentrationerna av makro- och mikronäringsämnen i grödorna påverkades främst med jordarna Kr och Nå som substrat till skillnad från Sä. Exakt vad det är hos vattenslammet som påverkade grödorna, och varför det såg olika ut på de tre jordarna, är svårt att precisera eftersom många andra faktorer hos jordarna (t ex struktur, organiskt innehål) påverkar vid tillsats av slammet.

Tillförsel av makro- och mikronäringsämnen i förhållande till koncentrationerna i jorden

I detta försök hade 15 respektive 30 ton avvattnat slam per hektar tillförts till jordarna, vilket är relativt låga mängder av vattenslam på åkermark. I odlingsförsöket motsvarar dessa mängder 47 gram respektive 92 gram avvattnat slam till kärlen som hade en area på 0,0314m^2 och volym på 5 L (ca 6 kg jord). Jämfört med mängden av N, P och K som tillfördes med gödslet var vattenslammets innehåll av N försumbart (tabell 1) och den växttillgängliga poolen av P och K var lägre respektive lika som i jordarna (tabell 3). Jämfört med de tre jordarna innehöll vattenslammet högre total-koncentrationer av S, Al och Cu (tabell 4 och 5) liksom något högre pH (tabell 3). Makro- och mikronäringsämnenas växttillgänglighet påverkades i hög grad av jordens pH, till exempel gav ett minskande pH även en minskad löslighet av Mo medan löslighet av Cu och Zn ökade. Ett minskat pH på grund av vattenslamtillförseln skulle ha kunnat förklara förändringarna av dessa ämnen i Italienskt rajgräs men de jordprover som togs vid försökets slut visade inte på någon säkerställd pH-ändring hos jordarna (tabell 10). Likaså visade jordproven som analyserats på växttillgängligt P, K, Mg, Ca och Fe (tabell 10) ingen påverkan från vattenslammet. Dessa siffror får dock jämföras med försiktighet eftersom dessa analyser gjordes på ett prov där de tre upprepningarna av varje behandling för varje jord slagits ihop. Samma tabell visar på en möjlig ökning av växttillgängligt Al på alla tre jordar på grund av vattenslamstillförseln men nivåerna var inte anmärkningsvärt höga jämfört med vad man brukar finna i Mellansverige.

Tillförseln av vattenverksslam på jordarna i relation till skördeeffekt och växternas innehåll av makro- och mikronäringsämnen

Det finns inte en enkel förklaring till att vattenslammet ökade skörden av Italienskt rajgräs på mellanleran Kr och sandjorden Nå medan den minskade skörden på lättleran Sä. Några skillnader att lägga märke till är de överlag högre poolerna av växtillgängligt P och K i Kr och Nå jämfört med Sä, gödslingen och slamtillförseln till trots, liksom det något högre pH på Nå (runt 6,6) och Kr (6,0) jämfört med Sä (5,4). Trots dessa goda grundförutsättningar och upprepad gödsling av NPK uppvisade kontrollerna av italienskt rajgräs odlad på Kr och Nå bristsymptom och gav lägre skörd än på Sä-jorden. Först med slamtillförsel var skörden på Kr och Nå i samma storlek som på kontrollen av Sä. Svavel är ett viktigt makroämne för grödor och i många områden i Sverige gödslas det med S. I detta försök ökade S koncentrationerna generellt i både Italienskt rajgräs och vårkorn på Nå och Kr på grund av vattenslamstillförsel. Dessa jordar hade också lägre total-S-koncentrationer än Sä, varför S från vattenslammet, i kombination med de högre växttillgängliga poolerna av P och K, kan vara en förklaring till ökade skörden av Italienskt rajgräs. Dessutom liknar bristsymptom av S (ljusa yngre blad) det för N och sålunda kan gödslingen med NPK, som gjordes för att häva bristsymptomen, ha förstärkt S bristen hos kontrollerna och den positiva effekten av S från slammet på skörden hos de behandlade leden. Detta kunde konstateras först efter att växterna skördats och därför gödslades det med lite mer NPK än brukligt i kärlförsök.

Förutom de faktorer som mättes i detta experiment finns det fler positiva aspekter av slamtillförsel som visats i andra studier, såsom ökad stabilitet av jordaggregat och ökad luftning liksom ökad aktivitet av mikroorganismerna, som lett till ökad skörd. Å andra sidan har även minskad skörd konstaterats som följd av vattenslamstillförsel. Däremot har kalkningseffekten, som mätts i andra experiment, inte visat sig här.

Utifrån dessa resultat kan man dra slutsatsen att låga givor vattenverksslam på jordar som liknar Kr och Nå inte bör ge några negativa effekter på grödorna. Men, innan storskalig spridning på åkrar kan rekommenderas bör fältförsök, som har mer variabla miljöer än växthusmiljön, testas.

Tack

Vi vill rikta ett varmt tack till Norrvatten som har finansierat denna studie.

Contents

1. Introduction

1.1 Drinking water residual sludge

In Sweden, approximately 1000 Mm^3 of drinking water are produced annually in water treatment plants (Jonasson, 1996). Of that amount, half the volume originates from groundwater and the other half from surface water. Aluminium sulphate ($Al_2(SO_4)_3$) is usually added during the drinking water treatment process in order to precipitate out organic and inorganic material (Bugbee & Frink, 1985). The resulting aluminium-based water treatment residual sludge (Al-WTR, hereafter referred to as WTR) is a gelatinous precipitate of aluminium hydroxide together with the organic and inorganic matter from raw water. Aluminium is found in all plants (Delhaize & Ryan, 1995) but becomes toxic when the soil pH falls below 5.5 (Foy et al., 1978). WTR usually contains smaller amounts of heavy metals and nutrients than sewage sludge (Grabarek & Krug, 1987). In addition, its pathogen and toxic organic substances are low (Elliott & Demsey, 1991). WTR is currently classified as non-hazardous in the European list of wastes (Code 190902) and there are no strict rules on its disposal (Babatunde & Zhao, 2007). However, since 2003 Swedish law no longer permits WTR to be deposited in lakes, e.g. Lake Mälaren (SEPA, 2003).

Macronutrients include nitrogen (N), potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P) and sulphur (S), while micronutrients include iron (Fe), boron (B), manganese (Mn), zinc (Zn), copper (Cu), molybdenum (Mo) and nickel (Ni). In addition to these micronutrients, other potential toxic trace elements such as mercury (Hg), lead (Pb), cadmium (Cd) and arsenic (As) are usually found in small amounts. This study examines most of these elements (K, Ca, Mg, P, S, Fe, Mn, Zn, Cu, Mo, Ni, Pb and Cd) but not those with analytical problems (Hg and B) (Robert & Ralf, 2009).

Suggested benefits of application of WTR to productive areas of land are that it can increase N availability for the plants, improve soil aggregate stability and oxygen conditions and make water infiltration more even (Ippolito et al., 2003). A study on application of WTR (0.1-10 g kg^{-1}) in a pot experiment showed that soil aggregation and water retention improved, as did yield of maize (*Zea mays* L.) (Rengasamy et al., 1980). However, when the application rate was increased, germination problems followed. At application rates of 20 and 100 g kg^{-1} WTR to a silt loam tomato (*Lycopersicon esculentum* L.) growth was reported to increase (Elliot & Singer, 1988). In a laboratory study examining the various extractable Al forms in WTR as a function of age, freshly generated WTR samples were potentially more reactive in stabilising the soil, but decreased in reactivity six months later (Agyin-Birikorang & O'Connor, 2009). Those authors concluded that the growth increase observed was due to a reduction in Al and Mn toxicity in the soil caused by the increase in pH from 5.3 to 8.0 brought about by the liming effect of WTR.

Different plant species and varieties differ in their need for macro- and micronutrients and, in addition, the minimum critical concentration of essential elements required for plant growth varies from one species to another (Epstein, 1965). Few studies have examined the amount of freshly generated WTR needed to achieve a good crop or whether there is any benefit of decreasing the trace elements in the sludge. There may be a combined effect of increased trace element concentrations and pH after WTR application, since trace element uptake in plant shoots has been demonstrated to decrease as a result of soil fixation at higher pH (Elliot & Singer, 1988).

The greatest concern has been about the reduced P availability that may follow WTR application. In pot experiments, addition of 0-670 g kg^{-1} WTR resulted in reduced P availability and reduced yield of lettuce (Bugbee & Frink, 1985). Similarly, Heil and Barbarick (1989) applied WTR at rates of 0-25 t ha^{-1} to sorgum-sudan grass (S*orghum bicolour* L.) and found that yield decreased after WTR addition at rates exceeding 15 t ha^{-1} as a result of P fixation by WTR. Another study by Skene et al. (1995) showed reduced growth of broad beans (*Vicia faba* L.) when WTR was evenly applied to a sandy surface layer at rates of 20, 40 and 100 g kg^{-1} (~ 20-100 t ha^{-1}).

Beside limited knowledge of trace element uptake, little is known about the effect of WTR application on soil microorganisms. An exception is a study carried out in South Africa, where WTR was added to two soils at various rates and soil respiration monitored. It was found that amending the soil in question with WTR significantly increased soil respiration. However, the effect declined after some weeks, which means that increased microbial activity may not be a long-term result (Pecku et al., 2006). The toxic levels of different elements vary greatly for ruminants and consequently specific standard requirements are needed for feed consumption (George, 2004).

1.2 Objectives and hypotheses

The overall objective of this study was to examine the effects on growth and concentrations of elements in two types of the plants used for animal feed of WTR application to three types of soils. In addition, plant element off-take was calculated for some elements. Macronutrients as well as trace elements (micronutrients and potential toxic elements) were studied.

Specific hypotheses were:

- Adding WTR to the soil will not significantly alter harvested biomass production in spring barley and Italian ryegrass.
- Adding WTR to the soil will not significantly alter trace element concentrations in harvested spring barley and Italian ryegrass.
- Adding WTR to the soil will not significantly alter macronutrient and trace element offtake in harvested spring barley and Italian ryegrass.

2. Materials and Methods

2.1 Pot experiment

A pot experiment was conducted in a greenhouse with three soils: A clay loam rich in P from Krusenberg (Kr), a loamy sand rich in P from Nåntuna (Nå) and a silty loam with a low soil P concentration from Säby (Sä). Two levels of WTR were applied to each soil and two crop species were tested, the fast growing forage species Italian ryegrass (*Lolium multiflorum* Lam. cv. Fredrik) and spring barley (*Hordeum vulgare* L., cv. Barbro). The pots had a volume of 5 L (surface area 0.0314 m^2) and each treatment was carried out in triplicate. The species were chosen to represent the two most commonly grown feed crops, cereals and forage.

Control pots without any WTR application but with each species on each soil were included. The pots were placed in a randomised block design.

The experiment lasted for 12 weeks. To speed up plant growth, the greenhouse climate was set to long, warm days, 20ºC during daytime for 20 hours and 15ºC at night for 4 hours. The grass was cut twice and the barley was harvested at seed filling. Only distilled water was used to water the plants.

2.1.1 WTR application and sowing of seeds

WTR was applied to the pots at rates equivalent to 15 and 30 t ha^{-1} wet weight and had a dry weight (DW) content of 23%. It was collected from Lovön on 17 August 2010 and dewatered with polymers. The WTR was weighed and mixed thoroughly with the soil before sowing seeds of Italian ryegrass (30 seeds per pot) and spring barley (14 seeds per pot). After germination, the young plants were reduced to 15 and 7 plants per pot for ryegrass and barley, respectively, which is equivalent to 480 and 2230 plants per m^2.

2.1.2 Fertiliser application

Pure chemicals (NH_4NO_3, K_2PO_4 and KCl) were used as N:P:K fertiliser in order to avoid contaminating the experiment with any additional elements (Table 1).

Table 1. *Nutrient load of N-P-K (kg ha^{-1}) through WTR and fertiliser application (kg ha^{-1})*

Nutrient	**WTR application**		**Fertiliser (kg ha^{-1})**
	15 kg ha^{-1}	**30 kg ha^{-1}**	
N	10.5	21	220
P	13.5	27	66
K	15	30	220

Table 2. *Particle size distribution (%) and soil texture class of the three soils used in this study*

Soils	**Clay**	**Silt**	**Sand**	**Texture**
Krusenberg (Kr)	38	40	22	Clay loam
Nåntuna (Nå)	10	9	81	Loamy sand
Säby (Sä)	20	56	24	Silty loam

Fertiliser was applied at a rate of 50:15:50 kg N:P:K ha^{-1} at the start of the experiment, after the first cut of the ryegrass (50:15:50 kg ha^{-1}) and thereafter when the plants in the control started to look light green, which might be a sign of nutrient deficiency. This occurred twice and although the pots with added WTR never showed any sign of nutrient deficiency, they were equally fertilised. In total, the plants received the equivalent of 220:66:220 kg N:P:K ha^{-1} (Table 1).

2.2 Soil collection, preparation and texture

The three soils (Table 2) were collected close to the city of Uppsala, Sweden. At all of these sites the covering turf was removed and only topsoil was taken (0-15 cm depth). All soils were sieved through a 20-mm mesh. The subsamples for analyses as well as the WTR were dried at 30ºC overnight. Each sample was then ground in a ceramic pestle and mortar to increase homogeneity. All equipment was washed and dried after each mixing to avoid contamination. The particle size distribution of the three soils was determined with the pipette method at the laboratory of the Department of Soil and Environment, SLU, and classified according to Davis & Bennett (1927) (Table 2).

2.3 Chemical analysis of soils, WTR and plants

A portion of each plant and soil sample was dried at 105°C according to Swedish Standard SS028113 for determination of dry matter. The first samples used for chemical analysis were dried at 50°C and the elemental concentrations were corrected to dry weight (DM) from dry matter content.

Soils and WTR before growing crops. Total N and C content of soils and WTR was measured by a high temperature induction furnace combustion method using LECO CN2000 (LECO Co-operation, 2003). Plant-available nutrients (Ca, K, Mg, P) were analysed after extraction with ammonium lactate (AL) extraction according to Egnér et al. (1960) and Swedish Standard (1993). The extraction solution consisted of 0.1M ammonium lactate and 0.4M acetic acid (pH 3.75). Final analysis was made by ICP (Spectro Flamme, Germany) at AGRILAB, Uppsala. Acid extractable ("semi-total") concentrations of a Al, Ca, Fe, K, Mg, P and S and the trace elements Cd, Co, Cr, Cu, Mn, Mo, Ni, Zn and Pb in soils and WTR were analysed at Dept. Soil and Environment, SLU. Approximately 5 g dry weight of soil material was transferred to a 50 mL digestion tube, 20 mL 7 M HNO_3 were added and the samples were boiled at 120ºC in two hours (SIS, 1997). Final analysis of the trace elements took place by ICP-MS and of the macronutrients by ICP-AES.

Soils after growing crops. On termination of the crop experiment, the soils in the pots used for growing Italian ryegrass were sampled (replicates were pooled) and analysed for plant available (AL) nutrients (Al, Ca, Cu, Fe, K, Mg, and P) at AGRILAB, SLU, using the Swedish standard SS028310 (1993). The same laboratory analysed acid (2 M HCl) extractable nutrients (Cu, K, P) at the end of the pot experiments using the Swedish method KLK 1965:1. For this, 2 g soil was weighed into a glass bottle, 50mL 2 M HCl were added and the mixture was boiled in a water bath for two hours. The samples were shaken after 30 minutes, then cooled and filtered through folded filter paper (Munktell V00A) into plastic bottles with lids. Elements in the soils were analysed using ICP-AES (Spectro Flame Germany). Analyses of pH were made for every pot used for growing Italian ryegrass, using 5 mL soil (dried at 30ºC) and 25 mL distilled water, shaken for 5 minutes (Eriksson et al., 2010).

Plants. Plant samples were analysed for Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P Pb, S Ti V and Zn at the commercial laboratory ALS Scandinavia at Luleå. After digestion with 7 M nitric acid (HNO_3) and 30% hydrogen peroxide (H_2O_2) in Teflon containers, the elements were analysed with inductive coupled plasma ICP-AES and ICP-SFMS (analysis package M-3, metals in biological material).

2.4 Characteristics of soils and WTR

Determination of plant-available macronutrients (Table 3) at the start of the experiment showed that Kr clay loam had higher concentrations of plant-available Mg and Ca than Nå loamy sand and Sä silty loam, while Nå loamy sand had higher amounts of P than Kr clay loam and Sä silty loam. The WTR had a higher concentration of Ca than the three soils.

Table 3. *pH and concentrations (g kg^{-1}) of plant-available macronutrients (according to the AL-method) in soils and WTR at the start of the experiment*

Soil	pH	K-AL	P-AL	Ca-AL	Mg-AL
Kr clay loam	6.0	0.2	0.084	2.0	0.2
Nå loamy sand	6.7	0.2	0.091	1.4	0.0
Sä silty loam	5.6	0.1	0.035	1.5	0.1
WTR	6.5	0.2	0.013	2.3	0.1

In terms of total concentration of macronutrients, Kr clay loam had higher concentrations of P, Na, Mg, K, Fe, Ca and Al than Nå loamy sand and Sä silty loam (Table 4). Sä silty loam had higher total concentrations of C, N and S than Kr clay loam and Nå loamy sand, whereas WTR had higher concentrations of N, C, S, Na and Al than the three soils, but a similar concentration of P.

The Kr clay loam generally had higher concentrations of the trace elements Pb, Cd, Mo, Zn, Co, Cu Ni, Mn and Cr than the Nå loamy sand and Sä silty loam, while Nå loamy sand had the lowest concentrations of these elements in most cases (Table 5). The WTR had a higher concentration of Cu than the three soils.

2.5 Harvest

The Italian ryegrass was cut twice, while the spring barley was harvested at seed filling. Both species were cut at 5 cm above ground level. The harvested biomass was sealed in perforated polythene bags and dried in a oven at 55ºC for 2 days, after which dry weight was measured. The samples were then milled in a Titanium mixer and placed in labelled plastic containers for analyses.

Table 4. *Concentration (g kg^{-1}) of macronutrients in soils and WTR digested with 7M HNO_3 at the start of the experiment*

Soil	**N ‘ %**	**C %**	**Al**	**Ca**	**Fe**	**K**	**Mg**	**Na**	**P**	**S**
Kr clay loam	0.1	2	22	7	30	6	8	0.3	1.0	0.2
Nå loamy sand	0.1	1	12	5	16	2	5	0.1	0.6	0.1
Säsilty loam	0.2	3	16	5	21	3	5	0.2	0.9	0.4
WTR	0.7	17	178	4	5	1	1	0.4	0.9	6.4

Table 5. *Trace element concentrations (mg kg^{-1}DM) in soils and WTR digested with 7M HNO_3 at the start of the experiment*

Concentration, mg kg^{-1}	**Cr**	**Mn**	**Ni**	**Co**	**Cu**	**Zn**	**Mo**	**Cd**	**Pb**
Kr clay loam	40	700	26	14	25	92	1.8	0.31	16
Nå loamy sand	13	330	8	5	12	43	0.3	0.10	10
Sä silty loam	25	300	17	9	14	50	0.5	0.13	12
WTR	7	410	22	2	35	16	0.9	0.04	4

2.6 Calculations and statistical analysis

In order to determine the amount of elements removed from the soil by the harvested biomass, the total offtake (kg ha^{-1}) of different elements (P, S and Cu) was calculated from biomass production (g DM) and concentration (mg kg^{-1} DM) based on the surface area of the pots. Statistical analysis of variance (ANOVA) was performed on the concentrations of elements in each harvest that were above the detection limit, using a computer software system (JMP; SAS). Similar statistical analyses were performed for the total offtake of P, S and Cu in Italian ryegrass and spring barley. Log transformations were performed on elements where residual plots were not normally distributed. Statistical analyses were also performed on harvested yield and pH between means of treatments within each species using Tukey's (HSD) test at a significance level of $p<0.05$.

3. Results

3.1 Harvested biomass

Harvest results from the different cutting regimes for Italian ryegrass (including combined harvest) and spring barley in the different soils and treatments showed a general increase in biomass production as a result of WTR application (Table 6).

The first and second cuts of Italian ryegrass had higher biomass production with the lower rate of WTR application than in the control on Kr clay loam. This led to an overall significant increase in combined harvested yield. However, there was no significant difference in biomass between the 15 and 30 t ha^{-1} WTR loads. Both first and second cut on Nå loamy sand were significantly affected by WTR application. Biomass yield was significantly higher with 30 t ha^{-1} WTR application compared with the control, but there were no differences between the two WTR application rates. Sä silty loam had significantly lower biomass production in the control than with 30 t ha^{-1} WTR application, while the load of 15 t ha^{-1} did not result in any significant change in biomass production when the three treatments were compared. In contrast to Italian ryegrass, there was no significant effect in biomass production on the three soils for spring barley.

Table 6. *Mean biomass production (g dw^{-1}) for the different cuts of Italian ryegrass and spring barley in the three different soils for the control (T0), 15 t ha^{-1} WTR (T1) and 30 t ha^{-1} WTR (T2) and their P-values*

Soil	Italian ryegrass Cut 1			Italian ryegrass Cut 2			Italian ryegrassCut 1 + Cut 2			Spring barley		
	T0	**T1**	**T2**	**T0**	**T1**	**T2**	**T0**	**T1**	**T2**	**T0**	**T1**	**T2**
Kr clay loam	5.8 B	7.4 A	7.5 A	7.1 B	12.3 AB	10.3 A	13.0 B	17.6 A	19.8 A	10.5	16.8	17.2
P-value	0.0429*			0.0140*			0.0047**			0.0965 ns		
Nå loamy sand	6.9 B	8.2 AB	8.9 A	5.9 C	10.8 B	13.1 A	12.8 C	19.0 B	22.1 A	22.8	31.3	29.1
P-value	0.0167*			0.0003*			0.0003***			0.1014 ns		
Sä silty loam	8.6 A	7.8 AB	7.0 B	13.5	14.2	14.7	22.1	22.0	21.6	13.9	18.7	10.0
P-value	0.0210*			0.1107 ns			0.5483 ns			0.1334 ns		

ns= not significant. p<0.05 i.e. (0.01-0.05) =* least significant. p<0.01 i.e. (0.001-0.01) =**. P<0.001 i.e (<001) = ***most significant. Levels marked with different letters are significantly different.

3.2 Concentration of macronutrients and trace elements in Italian ryegrass

The chemical composition of the first cut of Italian ryegrass within each soil and treatment is summarised in Table 7a. Nickel was below the detection limit in Italian ryegrass grown on Sä silty loam and Nå loamy sand, while Mo was below detection limit in Italian ryegrass grown on Sä silty loam. The concentrations of Ca, Fe, K, Mg, Mn, Na, Ni, P and Zn in Italian ryegrass did not show any significant differences due to WTR application on any of the three soils.

3.2.1 Concentration of macronutrients and trace elements in Italian ryegrass first cut

Krusenberg clay loam. When Italian ryegrass was grown on Kr clay loam, WTR application had a significant effect on plant concentrations of Cu, Mo, and S (Table 7a). The concentrations of Cu and S showed a significant change with 30 t ha^{-1} WTR compared with 15 t ha^{-1} and the control. WTR application at 15 t ha^{-1} and 30 t ha^{-1} gave a higher Cu concentration in ryegrass than the control, while there was no difference between the two WTR loads. Moreover, there was a significantly higher plant S concentration as a result of WTR application. For any one soil type, the concentration of Mo in Italian ryegrass was lower with WTR application but there was no difference between the two WTR loads. Plant concentrations of Ca, Fe, K, Mg, Mn, Na, Ni, P and Zn were not significantly affected (see Appendix for details).

Table 7a. *Mean concentration (mg kg^{-1} DW) of Cu, Mo, Ni and S with no WTR application (T0) and application of 15 t ha^{-1} (T1) and 30 t ha^{-1} (T2) in Italian ryegrass first cut. Significant differences in concentration after WTR application are indicated*

	Kr clay loam			***Nå loamy sand***			***Sä silty loam***		
	T0	T1	T2	T0	T1	T2	T0	T1	T2
Cu	7.2 $_B$	8.6 $_A$	9.3 $_A$	7.7	8.8	7.6	8.9	9.1	8.5
P value		0.0018**			0.3779 ns			0.5179 ns	
Mo	28 $_A$	17 $_{AB}$	9 $_B$	3.6	3.9	3.1	<2	<2	<2
P value		0.0143*			0.1676 ns			-	
Ni	3.0	3.6	3.9	<2	<3	<3	<2	<3	<3
p value		0.3861 ns			-			-	
S	1020 $_C$	1760 $_B$	2360 $_A$	970 $_B$	1920 $_A$	2190 $_A$	2960	3610	3660
P value		0.0002***			0.0198*			0.0788 ns	

ns= not significant. p<0.05 i.e. (0.01-0.05) =* least significant. p<0.01 i.e. (0.001-0.01) =**. P<0.001 i.e (<001) = ***most significant. Levels marked with different letters are significantly different.

*Nåntuna loamy sand***.** When Italian ryegrass was grown on Nå loamy sand, WTR application gave a significant increase in the plant concentration of S. There was no significant difference between the 15 t ha^{-1} and 30 t ha^{-1} WTR loads. Plant concentrations of Ca, Cu, Fe, K, Mg, Mn, Mo, Na, P and Zn were not significantly affected. Nickel was below the detection limit.

Säby silty loam. When grown on Sä silty loam, Italian ryegrass showed no significant difference with WTR application for all elements mentioned above.

Second cut Italian ryegrass grown on any of the three soils showed no effect of WTR application in terms of plant concentrations of Cu, Fe, Mn, and P. Nickel was below the detection limit (Table 7b).

3.2.2 Concentration of macronutrients and trace elements in Italian ryegrass second cut

Concentrations of micronutrients and trace elements in the second cut of Italian ryegrass were generally the same as for the first cut with a few exceptions. Ni was below the detection limit in all three soils. Plant concentration of Na increased due to sludge application on Sä silty loam. Plant concentration of Cu was not significantly altered for all soils, while plant concentration of Ca, Zn and Mg decreased due to WTR application on all three soils. No significance differences were observed between the two WTR loads,. Plant concentration of K was significantly increased due to WTR application on all three soils, but there were no significant differences between the two WTR loads.

Table 7b. *Mean concentration (mg kg^{-1} DW) of Ca, K, Mg, Mo, Ni, S, and Zn with no WTR application (T0) and application of 15 t ha^{-1} (T1) and 30 t ha^{-1} (T2) in Italian ryegrass second cut. Significant differences in concentration after WTR application are indicated*

	Kr clay loam			Nå loamy sand			Sä silty loam		
	T0	T1	T2	T0	T1	T2	T0	T1	T2
Ca	6940 $_A$	5500 $_B$	5680 $_B$	8440	7300	7870	7760	7720	7260
P value		0.0184*			0.1155 ns			0.7911 ns	
K	44100 $_B$	48200 $_A$	49200 $_A$	41800 $_B$	48900 $_{AB}$	53100 $_A$	46100	52000	46300
P value		0.0141*			0.0348*			0.4811 ns	
Mg	3210 $_A$	2520 $_B$	2500 $_B$	2900 $_A$	2150 $_B$	2360 $_B$	3400	3400	3130
P value		0.0339*			0.0028**			0.4335 ns	
Mo	38c	18 $_B$	11$_A$	4	3	3	<2	<3	<2
P value		0.0143*			0.3129 ns			-	
Na	96.9	104.2	98.5	bdl	bdl	bdl	166.3 $_B$	191.7 $_B$	271.7 $_A$
P value		0.8370 ns			-			0.0120*	
Ni	<2	<3	<3	<2	<3	<3	<2	<3	<3
p value		-			-			-	
S	910 $_B$	1070 $_{AB}$	1240 $_A$	530 $_B$	1020 $_A$	1400 $_A$	1330 $_B$	1963 $_{AB}$	2317 $_A$
P value		0.0473*			0.0016**			0.0133*	

ns= not significant. p<0.05 i.e. (0.01-0.05) =* least significant. p<0.01 i.e. (0.001-0.01) =**. P<0.001 i.e. (<001) = ***most significant. Levels marked with different letters are significantly different.

3.3 Concentration of macronutrients and trace elements in spring barley

Spring barley when grown on any of the three soils showed no effect of WTR application in terms of plant concentrations of Ca, Cu, Fe, K, Mg, Mn, and Na (Table 7c). However, there were some significant differences for the elements P, S and Zn (see also Appendix).

*Krusenberg clay loam***.** When spring barley was grown on Kr clay loam, there was no sign of altered plant concentrations for any of the elements.

Nåntuna loamy sand. When spring barley was grown on Nå loamy sand, there was a significant difference in the concentrations of P, S and Zn. The plant concentration of P decreased as a result of WTR application compared with the control. There was no significant differences between the 15 and 30 t ha^{-1} WTR loads. There was a significant difference for S, with increased concentrations of S with increasing WTR load. There was a decrease in plant concentration of Zn for the 15 and 30 t ha^{-1} WTR loads compared with the control. Spring barley showed no sign of altered plant concentrations for P, S and Zn or any other elements for Nå loamy sand.

*Säby silty loam***.** Spring barley showed no sign of altered plant concentrations for any element analysed when grown on Sä silty loam

Table 7c. *Mean (three replicates) P, S and Zn concentration (mg kg^{-1} DW) with no WTR application (T0) and application of 15 t ha^{-1} (TI) and 30 t ha^{-1} (T2) in spring barley grown in the three soils. Significant differences in concentration after WTR application are indicated*

	Kr clay loam			***Nå loamy sand***			***Sä silty loam***		
	T0	**T1**	**T2**	**T0**	**T1**	**T2**	**T0**	**T1**	**T2**
P	6140	5140	4590	5470 A	4070 B	4100 B	3880	3620	3310
P value		0.3198 ns			0.0283*			0.5280 ns	
S	920	1120	1360	610 C	990 B	1260 A	1900	1700	2000
P value		0.0851 ns			0.0017**			0.3415 ns	
Zn	37	28	26	20 A	14 C	16 B	24	20	23
P value		0.3170 ns			0.0011**			0.3070ns	

ns= not significant. p<0.05 i.e. (0.01-0.05) =* least significant. p<0.01 i.e. (0.001-0.01) =**. p<0.001 i.e. (<001) = ***most significant. Levels marked with different letters are significantly different.

3.4 Offtake of P, S, Cu and Ni in Italian ryegrass and spring barley

Offtake was calculated for the four elements P, S, Cu and Ni, which were present in concentrations above the detection limit for Kr clay loam (Table 8). The offtake of P by Italian ryegrass was significantly higher for the 30 t ha^{-1} WTR application compared with the control. The 15 t ha^{-1} WTR load did not give any significantly enhanced offtake compared with the control or the 30 t ha^{-1} load. Sulphur offtake in Italian ryegrass was significantly higher for all treatments compared with the controls. Copper offtake in Italian ryegrass was also significantly higher with WTR application compared with the control, but not between the two levels of WTR application.

For Nå loamy sand, P showed significantly increased offtake after WTR application, but there was no significant difference in offtake between the two WTR loads. Sulphur offtake increased significantly with increased WTR application. Similarly, Cu offtake increased significantly in all WTR treatments compared with the control, but there was no significant difference in Cu offtake between the two WTR loads. Sä silty loam showed no significant effect on P offtake after WTR application, but a significant increase in S offtake. There was no significant difference in S offtake between the two WTR loads. Copper offtake showed no significant effects of WTR application.

A similar calculation of P, S and Cu offtake was made for spring barley (Table 9). Regarding Kr clay loam, there was no significant difference in P offtake, but S showed a significant increase in offtake with increased WTR application. Copper had a higher offtake with WTR application compared with the control. There was no significant difference in Cu offtake between the two WTR loads.

Table 8. *Total offtake of P, S, Cu and Ni (kg ha^{-1}) in Italian ryegrass with no WTR application (T0) and application of 15 t ha^{-1} (T1) and 30 t ha^{-1} (T2)*

Element	P			S			Cu			Ni		
Treatment	**T0**	**T1**	**T2**	**T0**	**T1**	**T2**	**T0**	**T1**	**T2**	**T0**	**T1**	**T2**
Kr clay loam *(mean)*	23.88_B	28.54_{AB}	30.81_A	3.92_C	7.63_B	10.39_A	0.028_B	0.041_A	0.049_A	0.007	0.007	0.012
p-value	0.0399*			0.0008***			0.0028**			0.1403 ns		
Nå loamy sand *(mean)*	23.79_B	33.32_A	38.81_A	3.12_B	8.51_A	12.09_A	0.027_B	0.045_A	0.050_A	-	-	-
p-value	0.0056**			0.0023**			0.0100*			-		
Sä silty loam *(mean)*	32.27	30.51	29.46	13.78_B	17.71_A	18.85_A	0.056	0.056	0.058	-	-	-
p-value	0.4710 ns			0.0069**			0.7038 ns			-		

ns= not significant. p<0.05 i.e. (0.01-0.05) =* least significant. p<0.01 i.e. (0.001-0.01) =**. P<0.001 i.e. (<001) = ***most significant. Levels marked with different letters are significantly different.

For Nå loamy sand, P did not show any significant change in offtake when WTR was applied. Sulphur showed a significant increase in offtake between the control and the two WTR application treatments. There was no significant difference in offtake between the two WTR loads.

In Sä silty loam, there was no significant effect on offtake for any of the three elements P, S and Cu.

3.5 Soil characteristics after last cut

At the end of the experiment, there were significant higher Al-AL concentrations in the soils that had received high loads of sludge compared to soils without such amendment (Table 10).. In addition, there was a tendency for lower K-AL, K-HCl and P-HCl concentrations in the Nå loamy sand and Sä silty loam with WTR addition. In contrast, there were no such tendencies for K-HCl and P-HCL in Kr clay loam which was the soil most rich in K and P from the start.

Table 9. *Offtake of P, S, Cu (kg ha^{-1}) in spring barley with no WTR application (T0) and application of 15 t ha^{-1} (T1) and 30 t ha^{-1} (T2)*

Element	P			S			Cu		
Treatment	**T0**	**T1**	**T2**	**T0**	**T1**	**T2**	**T0**	**T1**	**T2**
Kr clay loam *(mean)*	20.98	26.91	24.91	3.06 $_C$	5.79 $_B$	7.39 $_A$	0.019 $_B$	0.029 $_A$	0.029 $_A$
p-value		0.3342 ns			0.0001***			0.0192*	
Nå loamy sand *(mean)*	39.63	40.62	37.69	4.44 $_B$	9.81 $_A$	11.62 $_A$	0.027	0.033	0.033
p-value		0.7079 ns			0.0009***			0.1914	
Sä silty loam *(mean)*	17.91	21.25	10.73	8.33	9.98	6.41	0.021	0.014	0.024
p-value		0.2173 ns			0.2391 ns			0.2238 ns	

ns= not significant. p<0.05 i.e. (0.01-0.05) =* least significant. p<0.01 i.e. (0.001-0.01) =**. P<0.001 i.e. (<001) = ***most significant. Levels marked with different letters are significantly different.

Table 10. *Concentration (g kg^{-1}) of plant-available (AL-digestion) macronutrients and extractable P, K and Cu (HCl-digestion) in bulk soil samples (no replicates) at the end of the experiment with Italian ryegrass*

Soil	P-AL	K-AL	Mg-AL	Ca-AL	Al-AL	Fe-AL	K-HCl	P-HCl	Cu-HCl
				Kr clay loam					
T0	0.10	0.24	0.20	2.28	0.15	0.34	4.5	0.80	0.24
T1	0.10	0.21	0.20	2.30	0.22	0.34	4.64	0.84	0.25
T2	0.10	0.22	0.21	2.33	0.28	0.30	4.56	0.85	0.25
				Nå loamy sand					
T0	0.10	0.19	0.06	1.59	0.14	0.13	1.70	0.54	0.10
T1	0.11	0.15	0.05	1.68	0.18	0.13	1.51	0.51	0.10
T2	0.10	0.13	0.06	1.64	0.25	0.12	1.36	0.49	0.10
				Sä silty loam					
T0	0.04	0.07	0.13	1.78	0.23	0.26	1.33	0.78	0.13
T1	0.04	0.07	0.13	1.80	0.28	0.27	1.20	0.71	0.13
T2	0.04	0.06	0.14	1.84	0.38	0.28	1.19	0.70	0.13
				Mean three soils					
T0	0.08	0.17	0.13	1.88	0.17	0.24	2.51	0.71	0.16
T1	0.08	0.14	0.13	1.93	0.23	0.25	2.45	0.69	0.16
T2	0.08	0.14	0.14	1.90	0.30**	0.23	2.37	0.68	0.16

** Significant (p<0.05) higher concentrations than for soil without AlWTR

3.5.1 pH analysis

pH did not change significantly after WTR addition to any of the three soils (Table 11).

Table 11. *pH values in the three different soils cropped with Italian ryegrass at the end of the experiment (significance levels shown)*

mean pH	T0	T1	T2
Kr clay loam	5.94	5.87	6.11
P-value		0.3947 ns	
Nå loamy sand	6.59	6.64	6.59
P-value		0.9465 ns	
Sä silty loam	5.37	5.54	5.46
P-value		0.1824 ns	

4. Discussion

4.1 Changes in biomass production

There was an overall improvement in yield of Italian ryegrass following WTR application on two of the soils, Kr clay loam and Nå loamy sand (Table 6). On the other hand, Italian ryegrass grown on Sä silty loam had decreased yield and spring barley did not show any significant changes in yield due to WTR application on any of the three soils.

The increased yield of Italian ryegrass on Kr and Nå could be a result of the physical and chemical properties of these soils (Agyin-Birikorang & O'Connor, 2009). The pH of the WTR was 6.5, which is within the general range of 5.0-8.0 suitable for plant growth (Bohn et al., 1985). Most nutrients are cations: Ca^{2+}, Mg^{2+}, K^{+}, NH^{4+}, Zn^{2+}, Cu^{2+} and Mn^{2+}. These cations are present in the soil solution and are also in dynamic equilibrium with the cations adsorbed on the surface of clay and organic matter, making them readily available for plant uptake.

Improved yield could also be a consequence of increased soil aeration as result of WTR application leading to increased microbial activity in the soils (Pecku et al., 2006). However, little is known about the impact of WTR application on soil microorganisms.

The increased yield could also be attributable to improved soil aggregate stability and oxygen conditions of the soil, as reported by Ippolito et al. (2003). Yield was reported to improve in another study as a result of better soil properties, such as aggregation and water retention (Rengasamy et al., 1980). In a similar pot experiment carried out by Elliot and Singer (1988), increased growth of tomato following WTR application was observed. The conflicting lack of improvement in yield of spring barley on Sä silty loam could be the result of the low pH of that soil, since values less than 5.5 usually increase Al toxicity to plants (Foy et al., 1978). However, no significant effects on the soils within treatments were noted when pH was analysed (Table 11). Thus it is not possible to identify with certainty the main reason for the lack of yield increase in barley following WTR application. Previous studies revealed that at WTR application rates higher than 670 g kg^{-1} soil, P was reduced and yield of lettuce was also reduced (Bugbee & Frink, 1985). In another study, Al-WTR applied at a rate of 25 g kg^{-1} soil to a sorgum-sundan grass crop decreased its yield (Heil & Barbarick, 1989).

4.2 Changes in trace element concentrations in spring barley and Italian ryegrass biomass

Total trace element concentrations (dissolved in 7M HNO_3) in the three soils were found to be below the permissible limits, which may imply that it is safe to apply this type of WTR to such soils (Eriksson et al., 2010). Likewise, trace element concentrations in the WTR were found to be under the critical limit in comparison to those given by the governmental prescriptions (SFS nr: 1998:944).

Plant concentrations of Cu (9.3 mg kg^{-1}) increased due to WTR application (Table 7a-c), but were below the range considered toxic to ruminants, which is 10-20 mg kg^{-1} (George, 2004).

Increased WTR application limited the concentration of Mo and Zn in Italian ryegrass and spring barley. Zn is found in every tissue in animals, but an excess in the diet causes depressed feed intake and induces Cu deficiency. However, ruminants have been found to have a relatively high degree of tolerance to Zn and direct toxicity is rare (George, 2004).

On the other hand, Mo and S has also been found to induce Cu deficiency, especially when the herbage level of S is at least 4,000 mg kg^{-1} DM, and in combination with herbage Mo concentrations above 3,000 mg kg^{-1} DM.

The high P concentration in the WTR was found to be decreased through offtake by Italian ryegrass in Kr clay loam (Table 8). Both Italian ryegrass and spring barley grown on Kr clay loam also took up a significant amount of S from the WTR. In Kr clay loam, which had a high concentration of Cu, Italian ryegrass was found to have a significantly lower concentration compared with spring barley (Tables 8 and 9). In similar experiments Baker & Senft (1995) observed that trace element offtake after application of NPK fertiliser was much lower than after WTR application. The difference in P offtake observed between greenhouse and field conditions has been attributed to the restricted volume of growing pots (Codling et al., 2007).

4.3 Changes in macronutrient and trace element offtake in harvested spring barley and Italian ryegrass

In this experiment the macronutrients N, P and K were applied as fertiliser and were not limiting for growth. However, S was found to be taken up significantly more at higher WTR application rates.

Offtake of Cu in Italian ryegrass was found to increase at moderate WTR application rates for Kr clay loam and Nå loamy sand (Table 8). This is in contrast with findings that both Cu and Ni showed a substantial reduction in uptake by tomato shoots as a result of WTR application (Elliot and Singer, 1988).

For WTR to be used in agriculture, it should be examined for both its short-term and long-term effects on soil quality. Continuous cultivation of the two species tested here on the same plot for some length of time may yield appropriate and more complete results clarifying the trends observed in the pot experiment. However, different authors have contrasting views on the use of WTR on arable land. Some studies report an improvement in water retention and pH, resulting in high crop yields (Rengasamy et al., 1980), while others have reported plant-available P in the soil to be reduced and crop yield depressed at high application rates (Young et al., 1988). A pot experiment does not provide sufficient data to conclude what happens in the field situation, when the plants is usually grown for a longer period of time.

4.4 Possible use of WTR for growing crops

Drinking water treatment residual sludge can be a source of micronutrients for Italian ryegrass and spring barley on soils similar to Nå loamy sand and Kr clay loam, since it has been proven to improve yield. The WTR could possibly be applied to agricultural soils with a high P-AL status (similar to Nå loamy sand and Kr clay loam) but only in moderate loads, since high loads will induce plant nutrient deficiency. However, further research is needed to determine whether there is a P limiting effect of WTR application on plant yield.

4.5 Summary

- In the present study, WTR application resulted in increased yield of Italian ryegrass on two of the three soils tested: clay loam and loamy sand. On the third soil type, silty loam, yield of Italian ryegrass was decreased.
- Yield of spring barley was not affected by WTR application.
- Higher amounts of the trace element Cu was taken off with harvested ryegrass and barley when WTR was applied to the studied clay loam and loamy sand.
- The WTR used in this study did not contain high concentrations of trace elements, i.e. micronutrients and potential toxic trace elements.

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Appendix 1

Mean concentration (mg kg^{-1} DM) of trace elements with no sludge application (T0) and application of 15 t ha^{-1} (T1) and 30 t ha^{-1} (T2) in Italian ryegrass first cut

Mean/ element	Krusenberg clay loam			Nåntuna loamy sand			Säby silty loam		
	T0	T1	T2	T0	T1	T2	T0	T1	T2
Ca	6937	6457	7420	8020	7537	6913	8717	8387	7810
P value		0.3316 ns			0.0513 ns			0.2643 ns	
Cu	7.21$_{B}$	8.57$_{A}$	9.27$_{A}$	7.67	8.75	7.64	8.88	9.08	8.54
P value		0.0018**			***0.3779 ns***			***0.5179 ns***	
Fe	62.10	65.07	71.77	52.10	62.93	59.93	76.73	88.73	80.10
P value		0.2843 ns			0.6816 ns			0.2269 ns	
K	45167	53367	53667	49767	56733	49500	51400	55300	53000
P value		0.0524 ns			0.3411 ns			0.5257 ns	
Mg	2257	2277	2520	1680	1750	1643	2893	2970	2320
P value		0.5912 ns			0.5891 ns			0.1414 ns	
Mn	70.17	72.07	67.63	22.37	28.10	26.00	88.97	84.27	77.00
P value		0.9124 ns			0.3059 ns			0.1176 ns	
Mo	27.60$_{A}$	17.27$_{AB}$	9.37$_{B}$	3.57	3.91	3.14	bdl	bdl	bdl
P value		0.0143*			0.1676 ns			-	
Na	104.67	146.33	156.00	100.63	100.27	84.33	397.33	440.33	424.33
P value		0.1131 ns			0.6893 ns			0.0932 ns	
Ni	2.98	3.56	3.89	bdl	bdl	bdl	bdl	bdl	bdl
p value		0.3861 ns			-			-	
P	5473	5420	4967	5363	5477	4870	4100	4043	4380
P value		0.2327 ns			0.4608 ns			0.4694 ns	
S	1017$_{C}$	1760$_{B}$	2363$_{A}$	966$_{B}$	1923$_{A}$	2187$_{A}$	2957	3610	3657
P value		0.0002***			0.0198*			0.0788 ns	
Zn	28.87	29.33	38.13	23.60	22.87	23.57	32.40	31.47	27.20
P value		0.0561 ns			0.9308 ns			0.1488 ns	

ns= not significant. p <0.05 i.e (0.01-0.05)=* least significant. p < 0.01 i.e (0.001-0.01)=**. P< 0.001 i.e (<001)= ***most significant. Levels marked with different letters are significantly different. bdl= below detection limit.

Appendix 2. Mean concentration (mg kg^{-1} DW) of trace elements with no sludge application (T0) and application of 15 t ha^{-1} (T1) and 30 t ha^{-1} (T2) in Italian ryegrass second cut

Mean/ element	Krusenberg clay loam			Nåntuna loamy sand			Säby silty loam		
	T0	T1	T2	T0	T1	T2	T0	T1	T2
Ca	6937 $_{A}$	5503 $_{B}$	5680 $_{B}$	8437	7300	7870	7763	7717	7257
P value	0.0184*			0.1155 ns			0.7911 ns		
Cu	6.35	6.30	6.98	5.41	6.47	6.90	7.50	7.99	8.04
P value	0.2494 ns			0.1836 ns			0.7124 ns		
Fe	76.13	68.30	71.37	49.27	68.20	71.37	82.53	83.93	84.63
P value	0.4890 ns			0.1541 ns			0.9643 ns		
K	44133 $_{B}$	48167 $_{A}$	49167 $_{A}$	41800 $_{B}$	48867 $_{AB}$	53100 $_{A}$	46067	52000	46267
P value	0.0141*			0.0348*			0.4811 ns		
Mg	3207 $_{A}$	2517 $_{B}$	2497 $_{B}$	2870 $_{A}$	2147 $_{B}$	2363 $_{B}$	3403	3403	3133
P value	0.0339*			0.0028**			0.4335 ns		
Mn	161.3	137.7	126.0	76.63	67.43	70.97	161.7	147.0	134.0
P value	0.1506 ns			0.4390 ns			0.4811 ns		
Mo	38.37$_{C}$	17.70 $_{B}$	11.22$_{A}$	3.54	3.07	3.01	bdl	bdl	bdl
P value	0.0143*			0.3129 ns			-		
Na	96.9	104.2	98.5	Bdl	bdl	bdl	166.3 $_{B}$	191.7 $_{B}$	271.7 $_{A}$
P value	0.8370 ns			-			0.0120*		
Ni	bdl	bdl	bdl	Bdl	bdl	bdl	bdl	bdl	bdl
p value	-			-			-		
P	5783	5063	5133	6370	5517	5953	4947	4590	4270
P value	0.2313 ns			0.0625 ns			0.5021 ns		
S	907.3 $_{B}$	1065 $_{AB}$	1236 $_{A}$	528.3 $_{B}$	1023 $_{A}$	1400 $_{A}$	1330 $_{B}$	1963 $_{AB}$	2317 $_{A}$
P value	0.0473*			0.0016**			0.0133*		
Zn	32.13	25.57	29.90	35.17 $_{A}$	26.23 $_{B}$	30.00 $_{AB}$	33.10	31.00	28.97
P value	0.3886 ns			0.0352*			0.4723 ns		

ns= not significant. p<0.05 i.e (0.01-0.05)=* least significant. p<0.01 i.e (0.001-0.01)=**. P<0.001 i.e (<001)= ***most significant. Levels marked with different letters are significantly different. bdl= below detection limit.

Appendix 3. Mean (three repetitions) concentration (mg kg^{-1} DW) of trace elements with no sludge application (T0) and application of 15 t ha^{-1} (TI) and 30 t ha^{-1} (T2) in spring barley grown

Mean/ element	Krusenberg clay loam			Nåntuna loamy sand			Säby silty loam		
	T0	**T1**	**T2**	**T0**	**T1**	**T2**	**T0**	**T1**	**T2**
Ca	3443	3740	3247	3947	3030	3420	2600	2433	2783
P value	0.7200 ns			0.3051 ns			0.8041 ns		
Cu	5.77	5.66	5.37	3.80	3.38	3.59	4.65	4.01	4.27
P value	0.8578 ns			0.1373 ns			0.3769 ns		
Fe	32.93	37.20	38.30	27.93	28.40	31.33	26.00	30.73	30.93
P value	0,5879 ns			0.6033 ns			0.4914 ns		
K	25467	28433	28567	29533	23467	29000	28567	26100	31967
P value	0.4988 ns			0.4019 ns			0.5714 ns		
Mg	2050	1817	1670	1897	1360	1400	1680	1647	1603
P value	0.5363 ns			0.1962 ns			0.9237 ns		
Mn	27.27	26.40	26.50	20.00	21.83	19.33	25.00	24.17	27.57
P value	0.9899 ns			0.8179 ns			0.7667 ns		
Na	253	280	274	99,1	113	123	506	533	532
P value	0.7312 ns			0.4907 ns			0.8713 ns		
P	6143	5143	4587	5473 $_A$	4073 $_B$	4097 $_B$	3877	3617	3307
P value	0.3198 ns			0.0283*			0.5280 ns		
S	920.3	1115.7	1360.0	614.0 $_C$	992.0 $_B$	1256.0 $_A$	1900.0	1696.7	2000.0
P value	0.0851 ns			0.0017**			0.3415 ns		
Zn	36.90	27.77	26.00	20.20 $_A$	13.90 $_C$	16.33 $_B$	24.33	20.40	23.30
P value	0.3170 ns			0.0011**			0.3070ns		

ns= not significant. p<0.05 i.e (0.01-0.05)=* least significant. p<0.01 i.e (0.001-0.01)=**. P<0.001 i.e (<001)= ***most significant. Levels marked with different letters are significantly different. bdl= below detection limit.