Is the Agricultural Utilisation of Treated Urine and Faeces recommendable?

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Keywords: Dry Toilet, Urine, Fertiliser, Pharmaceutical Residues, Earth worms, Parasites.

ABSTRACT

One big advantage of source control sanitation, like dry sanitation, is the possibility of fertiliser re-utilisation in agriculture. Due to limited fossil resources of phosphate this is needed to aliment people in the future. The utilisation of treated faecal matter and urine can help communities with limited economical resources to produce food more efficiently. Beside the ecological, economical and social benefits, there are remaining some risks. This paper presents result of accumulation of pharmaceutical residues in plants fertilised with urine and of an “earth worm avoidance response test” on different media produced in resources orientated sanitation.

INTRODUCTION

The resulting material from dry sanitation has a great potential to substitute mineral fertiliser in agriculture and to enrich soil with organic matter. The great advantage for units with urine diversion is the separation of pathogens and most of the parasites from the fertiliser substances. But a major part of the pharmaceutical residues and in particular water soluble substances are excreted via urine. Many of those do not show good biodegradability. Therefore, a database was set up with data from literature to analyse the behaviour of pharmaceutics in urine and the environment (www.tuhh.de/aww/pharma/) [1,2] and greenhouse experiment with rye grass were conducted [3].

The appearance of earth worm in the fertile top soil is considered favourable for the plant growth and health and is an indicator for a healthy soil environment. The reduction of earth worms due to human activities like manuring or ploughing is considered as unfavourable. To study the behaviour of earth worms in the presence of fertiliser product from resources orientated sanitation, an experiment was set up to investigate the preferences and avoidances of earth worms regarding these fertilisers. Different commercial mineral fertilisers as well as fertilisers from urine, blackwater and dry toilet matter were compared.

Moreover, many developing countries are suffering from a lack of health services, awareness and educational possibilities, beside other elementary things like food, houses and adequate

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1 The greenhouse experiments were conducted in cooperation with Joachim Clemens, Department Plant Nutrition, INRES, University of Bonn, 53115 Bonn, Germany
income. For this reason many organisations are promoting dry toilet or other “ecosan” systems to reduce these lacks. This is thought to be very positive, but the fact that many people are seriously affected by parasites is easily underestimated due to the lack of experience in Northern countries. To avoid the spread of parasites due to the use of faecal matter form dry toilets, a proper qualified treatment is required.

**METHODS**

To obtain an overview of the relevance of pharmaceutical residues in water bodies and excreta, data from literature and own data where collected and fed into a database [4]. This allowed to receive statistical data for the active agents if sufficient information could be collected. Due to the web interface [5] the public has access to the database as well. For the pot experiments three substances were selected: Carbamazepine CZ (anti epileptic), Ibuprofen IBU (analgesic) and 17α-Ethinylestradiol EE (contraceptive) that were found in human urine. Finally for the green house experiments the pots with rye grass were fertilised with spiked male urine. The concentration range of the pharmaceuticals was chosen as expected values in Middle Europe urine as well as enhanced by the factor 10 (EE by factor of 40).

The main idea is, to implement an easy applicable bio-test for fertilisers from urine and faeces. As organisms Earth worms (*Eisenia fetida*) were selected as organisms. Four buckets were placed, filled with garden soil and covered with soil in a way that all buckets were connected with each other via soil. 1 g N from urine, ammonia solution or mineral fertiliser spiked with 10 mg 10,11-Dihydrocarbamazepine, 50 mg Diclofenac sodiumsalt and 100 mg 2-(4-Chlorophenoxy)-2-methylpropionic acid dissolved in 500 ml water was applied to each bucket. This amount corresponds with the normal fertiliser dose of 150 kg N /ha. As reference a mineral N,P,K fertiliser was chosen. 50 to 100 earth worms were placed in the mid between the four buckets. 24h later the worms were counted in every bucket. To become statistical sure, every experiment was repeated 9 to 10 times. Figure 1 shows the experimental set-up and a photograph respectively.

![Figure 1. Experimental set-up of the “Earth-worm Avoidance Response Test”](image-url)
RESULTS

Querying the above mentioned database for pharmaceuticals in the wastewater type “yellowwater” (urine) 17 components were found manifold. This seems to be little, but the main focus of analyses of pharmaceutical was set on surface water (drinking water supply) and the effluents of wastewater treatment plants. The result of the query is given in Figure 2. The three pharmaceutical chosen for the pot tests are included and marked orange. The analysis of pharmaceuticals in soil and plant parts is quite tricky. The limits of quantification (LOQ) of the TUHH central Lab for the three pharmaceutical are given in Table 1. The concentration of 17 α Ethinylestradiol is two low for the disposable analytic no effects could be determined.

Figure 2. Concentration of pharmaceutical substances in German urine obtain from a query of the database with values from literature (www.tuhh.de/aww/pharma).

Table 1. Limit of Quantification at TUHH Central Lab [3]

<table>
<thead>
<tr>
<th></th>
<th>LOQ in soil (µg kg(^{-1}) DM)</th>
<th>LOQ in roots (µg kg(^{-1}) DM)</th>
<th>LOQ in leaves (µg kg(^{-1}) DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ibuprofene</td>
<td>2</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>17 α Ethinylestradiol</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>0.6</td>
<td>20</td>
<td>75</td>
</tr>
</tbody>
</table>

Even with the 10 fold dosage of natural concentrations in urine, no effect on the biomass production could be observed. After the growth period of 92 days, only carbamazepine could be found in the soil of the pots. Because of the difficult matrix only carbamazepine could be detected and quantified in the soil and in the plant parts as well. About 50% of the carbamazepine could be found after 92 d in soil. In Figure 3 the concentration of carbamazepine (CZ) in soil, root and leavers of pot tests with rye grass applied 10 fold than natural concentration level sole and in combination with other pharmaceuticals after 92 days are given. Every test was conducted three fold and the standard deviations are given.
additionally. It is clearly to make out that carbamazepine is enriched in the root at factor 15 and in the leaves at factor 350 in relation to the soil concentration. The combination with other pharmaceutical substances remains unclear due to the deviation of the measured concentrations, but the general evidence of the enrichment in the plant is not effected by this. Ibuprofene was not found in soil after 92 day, supposedly because ibuprofene is degraded in the soil during this period.

Figure 3. Concentration of carbamazepine (CZ) in soil, root and leavers of pot tests with rye grass applied 10 fold than natural concentration level sole and in combination with other pharmaceuticals (Data from [3]).

The determination of toxic or other negative effects on plants can not be deduced from the existence of pharmaceuticals in plants. Therefore, an earth worm avoidance response test, as an easy an cheap method to determine effect on earth organism. *Eisenia foetida* was selected as indicator organism because it is wildly spread in Europe and easily to raise.

Figure 4. Result of the first run with 10 repetitions of the worm avoidance response test.

Figure 4 shows the results of the first run with 10 repetitions of the earth-worm avoidance response test. In the 4 bins mineral fertiliser was tested as reference with ammonia solution, to see if the ammonia is uncomfortable for earth worm, mineral fertiliser spike with the artificial pharmaceutical mixture described above and stored urine. The deviation, given as interval of
confidence at 95%, is flashy big, but in any case the ammonia solution is most popular to earth worms, while urine is most unpopular. Only half of the amount of earth worm were found in the urine fertilised compartment. The pharmaceutical did not have an effect on *Eisenia fetida*.

Due to the good acceptance of ammonia solution, sodium chloride was added for the second run, to see if the high salt concentration is the reason for the poor acceptance of urine by earth worm. Additionally beside urine, biological treated urine with nitrification was used for the test (Figure 5). In this case the compartment, fertilised with the ammonia solution, indicated the best acceptance for earth worm. The hight salt content was not the reason why worms avoid a compartment as assumed after the first run. Differently to the first run (10 repetitions), urine is accepted equal to mineral fertiliser in the second run (9 repetitions), while biological treated urine, with Nitrate as N-Source was unpopular for the worms.

![Figure 5](image_url) Result of the second run with 9 repetitions of the worm avoidance response test.

The two run were subsumed in Figure 6. The results with mineral fertiliser and ammonia solution show a good conformity while the tests with stored urine differed widely.

![Figure 6](image_url) Radar plot of the two run for the worm avoidance response test

**DISCUSSION AND CONCLUSIONS**

Querying the database for the appearance of pharmaceutical in plants many data could be found. But in general veterinary pharmaceuticals could be found. It is clear that
Biodegradability is the best option to prevent accumulation of pharmaceutics in plants. But it is not very likely that future pharmaceutics substances are designed more biodegradable. Therefore, more information about the behaviour of pharmaceutics is required to ensure the safety utilisation of urine as fertiliser supplement. The example in this study show an accumulation of carbamazepine by a factor of nearly 400 in the leaves, i.e. over 30% of the applied carbamazepine was found in the leaves of the rye grass. But an effect for consumers cannot be deduct at the moment.

The first test runs of the earth worm avoidance response test show a little effect on urine. Stored urine, treated and untreated is not as popular for the earth worm *Eisenia fetida* as ammonia solution with or without sodium chloride as well as mineral fertiliser. This result differs from Muskolus [6] observations, in which no worm were found 24 h after fertilising with urine. But the avoidance of worms do not mean that urine is less valuable as fertiliser than other products. These tests were performed with a soil for horticulture that is rich in organics. Due to good adsorption capacities the effect of unpleasant components in urine do not obtrude explicitly. Therefore, additional test with soil that is poor in organics have to be carried out.

ACKNOWLEDGEMENTS

The author like to thank the DBU (German Federal Foundation for the Environment), the Linz AG and the TUHH, especially the central lab (Dr. Reich and Mrs. Engel) for supporting this study. The research related to pharmaceutical substances was accomplished in the 6th EU framework project ‘SWITCH’. The plant trials were conducted in cooperation with Dr. Joachim Clemens, INRES, University of Bonn.

REFERENCES

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Institute of Wastewater Management and Water Protection
Presentation Contents

➔ Introduction
➔ Pharmaceutical residues in the environment
➔ Biotest: implemented as worm Avoidance Response Test
➔ Parasites in urine and faeces
➔ Conclusion
Introduction

➔ Intake of pharmaceutical is a daily routine
➔ Urine is an important fraction of resources orientated sanitation systems
➔ Urine contains the main part of fertiliser products and has a great potential for agricultural usage
➔ Urine contains pharmaceuticals
Set-up of a Database

Article → Location → Substance

Medium:
- Water, Wastewater, Soil, Plant

Degradation:
- i.a. Sorption, biolog. degradation

Metabolites
Web Interface of the Database

http://www.tuhh.de/aww/pharma/
# Querying the Database for Pharmaceuticals in the Environment

**Extended statistical evaluation of the query 'Appearance'**

**Version:** June 19th, 2008

Martina Winker and Joachim Behrendt

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<thead>
<tr>
<th>Choose</th>
<th>Chosen</th>
</tr>
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<tbody>
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Results
# Querying Results

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<th>Max</th>
<th>Average</th>
<th>Standard deviation</th>
<th>Cl 95%</th>
<th>Unit</th>
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<td>2.08e+04</td>
<td>6.41e+03</td>
<td>2.24e+03</td>
<td>ng/l</td>
</tr>
</tbody>
</table>

New Query
Concentration of Pharmaceuticals in Human Urine

- Ibuprofen (IBU)
- Ethinylestradiol (EE)
- Carbamazepine (CZ)
- Sitosterol (B-)
- Phenazone (pharmaceutical)
- Phenacetin
- Pentoxifylline
- Mestranol
- Indomethacin
- Fenoprofen
- Fenofibrat
- Estrone
- Estriol
- Estradiol, (17β)-
- Diclofenac
- Clofibric acid
- Bezafibrate

Concentration of Pharmaceutical S_PH [ng/l]
Pharmaceuticals found in Plants

- Erythromycin
- Sulfadimethoxine
- Tylosin
- Amoxycillin
- Doxycycline
- Chlorotetracycline
- Sulfadiazine
- Sulfamethoxazole
- Enrofloxacin
- Sulfamethazine
- Tetracycline
- Oxytetracycline
- Florfenicol
- Trimethoprim
- Diclofenac
- Sulfadiazine
- Pharamceutical Substance

Concentration in plants [ng/kg]
Pot Tests with Rye Gras

Selected pharmaceuticals: Carbamazepine (CZ), Ibupofene (IBU), 17α-Ethinylestradiol (EE)

Fertilisation 2 weeks after seeding

Two concentration level:
- natural (n)
- artificial (a): CZ & IBU: 10 x n EE: 40 x n

Male Urine
## Analytical Limitations

Limit of Quantification at TUHH Central Lab (Reich and Engel)

<table>
<thead>
<tr>
<th></th>
<th>LOQ in soil (µg kg(^{-1}) DM)</th>
<th>LOQ in roots (µg kg(^{-1}) DM)</th>
<th>LOQ in leaves (µg kg(^{-1}) DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ibuprofenene</td>
<td>2</td>
<td>30</td>
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<tr>
<td>17 α Ethinylestradiol</td>
<td>2</td>
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<tr>
<td>Carbamazepine</td>
<td>0.6</td>
<td>20</td>
<td>75</td>
</tr>
</tbody>
</table>
Result of Pot Tests

- 50% of the applied CZ could be found after 92 d
- IBU is beneath detection level in all compartments after 92 d
- EE is beneath detection level
- no influence on germination, growth and biomass production detected
Result of Pot Tests

Concentration in roots $C_{\text{CZ}}$ [µg/(kg DM)]

- CZ(a)
- CZ/IBU (a)
- CZ/EE (a)
- CZ/IBU/EE (a)

Concentration in leaves $C_{\text{CZ}}$ [µg/(kg DM)]

- CZ(a)
- CZ/IBU (a)
- CZ/EE (a)
Result of Pot Tests

- Accordance with literature due to degradation in soil: CZ 50% degradation, IBU degraded to a large extent
- Biological Degradation is the determining factor
- No effect on biomass production
- CZ enriched in root 20-fold in relation to soil and in leaves 400-fold in relation to soil
- It seems that persistent pharmaceuticals are enriched in plants
Earth Worm Avoidance Response Test

- The aim is to implement a bio test that can conduct easily.
- A typical earth organism is chosen that can be identified without expensive equipment.
- A soil for horticulture was chosen that contains a high content of organics.
- The soil could be bought at a garden market in a constant quality.
- Additional tests with organic poor earth will be performed later (no results in this study).
Experimental Set-up of Earth Worm Avoidance Response Test
Experimental Set-up of Earth Worm Avoidance Response Test
Experimental Set-up of Earth Worm Avoidance Response Test
Results of Earth Worm Avoidance Response Test

First run with 10 repetitions

Pharmaceutical mixture
Urine
Ammonia solution
Mineral fertiliser

Preference by worms [%]

First run with 10 repetitions
Results of Earth Worm Avoidance Response Test

First run with 10 repetitions

- Pharmaceutical mixture 26%
- Mineral fertiliser 25%
- Urine 14%
- Ammonia solution 35%
Results of Earth Worm Avoidance Response Test

Second run with 9 repetitions

- Mineral fertiliser
- Ammonia solution
- Urine
- Biol. Treated Urine

Preference by worms [%]
Results of Earth Worm Avoidance Response Test

Second run with 9 repetitions

- Biol. Treated Urine 19%
- Urine 25%
- Ammonia solution 32%
- Mineral fertiliser 24%
Results of Earth Worm Avoidance Response Test

Pharmaceutical mixture

Biol. Treated Urine

Mineral fertiliser

Stored urine

Ammonia solution

1 run

2 run
Worm Avoidance Response Test with Faecal Matter

Run with 5 repetitions

Data from Buzie

Run with 5 repetitions

Data from Buzie
Results of Earth Worm Avoidance Response Test

→ The earth worm have a small preference to mineral/ammonia fertiliser
→ Urine and biological treated urine is accepted by earth worm
→ Experiments show a big variance
→ The tree tested pharmaceuticals (Carbamazepine, Diclofenac, Clofibric acid) have no effect on worm behaviour
→ Urine typical salts concentration don't have an influence
→ Faecal matter is accepted by earth worms
# Patogenes and parasites

## Pathogen survival

*(time in days unless otherwise indicated)*

<table>
<thead>
<tr>
<th>Organism</th>
<th>Freshwater</th>
<th>Saltwater</th>
<th>Soil</th>
<th>Crops</th>
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<tbody>
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<td>Viruses</td>
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<td>&lt;10</td>
<td>15-100</td>
<td>5-50</td>
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<td>+285</td>
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<td>Fecal coliforms</td>
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<td>1.5 yr</td>
<td>2*</td>
<td>1-2 yr</td>
<td>&lt;60</td>
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<td>Tapeworm eggs</td>
<td>63*</td>
<td>168*</td>
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<td>Trematodes</td>
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<td>130**</td>
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</tbody>
</table>

ND No data

* Not considered an important transmission pathway

** Aquatic macrophytes
# Patogenes and parasites

## Pathogen survival

*(time in days unless otherwise indicated)*

Rose and Slifko 1999; Schwartzbrod 2000

<table>
<thead>
<tr>
<th>Organism</th>
<th>Freshwater</th>
<th>Saltwater</th>
<th>Soil</th>
<th>Crops</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viruses</td>
<td>11-304</td>
<td>11-871</td>
<td>6-180</td>
<td>0.4-25</td>
</tr>
<tr>
<td>Salmonellae</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>15-100</td>
<td>5-50</td>
</tr>
<tr>
<td>Cholera</td>
<td>30</td>
<td>+285</td>
<td>&lt;20</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Fecal coliforms</td>
<td>&lt;10</td>
<td>&lt;6</td>
<td>&lt;100</td>
<td>&lt;50</td>
</tr>
<tr>
<td>Protozoan cysts</td>
<td>176</td>
<td>1 yr</td>
<td>+75</td>
<td>ND</td>
</tr>
<tr>
<td>Ascaris eggs</td>
<td>1.5 yr</td>
<td>2*</td>
<td>1-2 yr</td>
<td>&lt;60</td>
</tr>
<tr>
<td>Tapeworm eggs</td>
<td>63*</td>
<td>168*</td>
<td>7 months</td>
<td>&lt;60</td>
</tr>
<tr>
<td>Trematodes</td>
<td>30-180</td>
<td>&lt;2</td>
<td>&lt;1*</td>
<td>130**</td>
</tr>
</tbody>
</table>

ND No data

* Not considered an important transmission pathway

** Aquatic macrophytes
Patogenes and parasites

http://curezone.com/image_gallery/parasites/
http://www.path.cam.ac.uk/partIB_pract/P15/

*Dracunculus medinensis*
Patogenes and parasites
Patogenes and parasites

Ascaris lumbricoides

Source of transmission:

➔ Soil and vegetation (faecal matter containing eggs deposited on the ground)
➔ Contaminated vegetable/water primary source of infection
➔ Municipal recycling of wastewater into crop field
➔ Eggs are concentrated in sludge by sedimentation
➔ Alkaline treatment inactivate eggs under some conditions
➔ 14°C and below, low inactivation rates with viable eggs for 6 months
➔ 24°C 6 months inactivation
➔ 34°C 1 month (Nordin et al 2009)
Schistosoma spp

- Schistosoma belong to the Trematode group and those infecting humans are colloquially known as blood flukes. During their life
- cycle schistosomas mature eggs are discharged with faeces into the water
- S. haematobium-urinary schistosomiasis
- S. mansoni intestinal schistosomiasis
Among water-borne pathogens, *Giardia* and *Cryptosporidium* are known to be highly resistant to water treatment procedures and to cause outbreaks through contaminated raw or treated water.

Giardiasis and cryptosporidiosis are also common infections of domestic and wild animals, which shed a large number of cysts and oocysts in the environment.

These cysts are insensitive to disinfectants at the concentration commonly used in water treatment plants to reduce bacterial contamination, although it has been shown that at higher concentrations of chlorine and ozone, *Giardia* cysts are less resistant than *Cryptosporidium* oocysts.

Moreover, *Giardia* cysts have been shown to survive in water for up to 2 months at temperatures as low as 8°C (26), and *Cryptosporidium* oocysts can survive for up to 1 year at 4°C in artificial seawater.

Numerous water-borne outbreaks of giardiasis and cryptosporidiosis have been documented in the past several decades, mainly in the United States, Europe, and Australia.

Conclusion

➔ Chemical and hygienic risks for reutilisation of toilet waste are still given

➔ but there is no reason to fall in panic

➔ High-tech approaches are available to solve this problem (Ozonisation for the liquid phase and Hydrothermal Carboxilation (HTC) for the sludge)

➔ At the moment low-tech solution are only possible with an integrated approach (health care, education, economical development...) and a proper management of the material (i.e. guaranty long storage time)
Thank You!
For your attention