A risk analysis of the potential harm on the soil environment caused by antibiotics in biosolids

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Abstract

The main purpose of this study was to make an initial risk analysis on the probability of antibiotics in biosolids causing adverse effects on agricultural soil environments. The issue on resistance development among bacteria towards antibiotics has also been discussed. Initially, the risk was found to be very high for all of the antibiotics; penicillin, tetracycline, macrolide, quinolone, trimethoprim and sulfonamide. The penicillins were, however, believed to be fairly easily degraded and therefore the risk they constitute might be considerably lower than was first estimated. The tetracyclines and quinolones were on the contrary found to be rather persistent, which strengthened the assumption of them constituting a very high risk. Sulfonamides were found to have a rather low potential to bind to sludge and the risk was therefore assumed to be somewhat overestimated for those drugs. Resistant bacteria have been found in sludge and the conditions in STPs have also shown to be suitable for the spreading of resistance among bacteria. It has further been found that plant uptake is a possible pathway for the resistant bacteria to reach humans. Hence, usage of biosolids on agricultural fields might have negative consequences.

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1.1. Background

Using sludge from sewage treatment plants (STPs) as a fertilizer on arable land has in the last decades become more and more common. The sludge contains a considerable amount of phosphorus and that is why it has such a great potential as a biosolid (sludge used as a fertilizer). Phosphorus is a limited natural resource and the will to recycle it from the STPs is therefore strong. In the year of 2005, it was decided by Swedish law that sewage sludge would no longer be allowed to be deposited on landfills and consequently, all the sludge had to be recycled (Naturvårdsverket, 2011). Most of the sludge has since then been used for different ground works or for landfill coverage and around 10 percent has been used on arable fields (Naturvårdverket, 2008). However, using sludge as a fertilizer is a very controversial issue. The main problem is that sludge contains many other substances, besides phosphorus, that might not be as good for the soil environment. The regulation of the content of biosolids does only concern metals (SNFS 1994:2), which makes the control over other substances poor. Pharmaceuticals are e.g. one group of compounds that are poorly controlled. They reach the STPs along with the wastewater after they have been excreted from the human body and within the STPs a considerable amount of the antibiotics end up in the sewage sludge (Lindberg et al., 2005, McClellan and Halden, 2010).

The kinds of pharmaceuticals that will be in focus in this study are the antibiotics. The concern regards the effect these compounds may have on the soil environment of agricultural fields. The most obvious concern relates to how the antibiotics will affect the bacteria in the soil, since the role of antibiotics are to kill bacteria. Other organisms may also be affected, either directly or indirectly by a disturbance in the ecosystem of the soil. However, negative effects on the soil ecosystem are not the only fear with antibiotics but also the risk for the development of resistance amongst the bacteria towards these compounds. The resistance can later spread to the bacteria causing human diseases (Kumar et al., 2005, Naturvårdsverket, 2005). The World Health Organization (WHO) considers the resistance of pathogenic bacteria a global threat to the human health and works actively to prevent the spreading (WHO, 2011). The work mainly consists of trying to stop unnecessary prescriptions of the drugs. The current situation in Sweden is not that bad. However, a widespread use of sludge as a soil fertilizer might change that. The yearly consumption of antibiotics in Sweden reaches around 400 tonnes (Naturvårdsverket, 2005). The most commonly used groups of antibiotics are penicillins, tetracyclines, macrolides, quinolones, trimethoprim and sulfonamides, with the penicillins being the most common (Apoteket, 2009, Apoteket, 2010). Some active substances that have been brought up in this study are not for sale any more in Sweden. They have, however, been used anyway to get enough data for the different calculations.

1.2. Aim

To deal with these issues of concern, the main part of this work has been to make an initial risk analysis for each of the antibiotic groups concerning the probability of them reaching concentrations in biosolids (from Swedish STPs) that could give rise to negative effects on the

soil ecosystem of agricultural fields. The persistence of the antibiotics in soil is an important factor for the evaluation of the risk and therefore the fate of the antibiotics after they have reached the soil has also been studied. The work also aimed to deal with the issue of resistance towards antibiotics among bacteria, which included a discussion of the current situation, of the ability for the spreading and of the seriousness of the matter.

1.3. The antibiotics

Penicillins act by inhibiting the formation of cell walls in bacteria, by interfering with the peptidase enzymes, which helps building up the cross linkage between peptidoglycan strands (Halling-Sørensen, 2001). The antibiotics are active against both gram negative and gram positive bacteria. Penicillin V (*fenoximetylpenicillin)* is the most common active substance in penicillin used by humans and affects a small spectrum of bacteria (Sjukvårdsrådgivningen, 2011). It is most frequently used for treating respiratory ill-health. Another big group is amoxicillin (fig.1), which is effective against a broader spectrum of bacteria. Other active substances of the penicillin group are e.g. penicillin G, mecillinam and ampicillin.

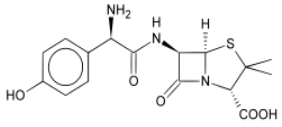

Figure 1: Amoxicillin

Tetracyclines affect the metabolism of the bacteria and thus prevent them from multiplying (Sjukvårdsrådgivningen, 2011). They do so by binding reversibly to the bacterial 30 S ribosomes, which induces an inhibition in protein synthesis (Halling-Sørensen, 2001). The tetracyclines are effective on both gram positive and gram negative bacteria (Halling-Sørensen, 2001) and are used for e.g. treatment of pneumonia, abdominal infections and sinusitis when penicillin, for some reason, cannot be used (Sjukvårdsrådgivningen, 2011). Some common active substances in tetracyclines are doxycycline, oxycycline and tetracycline (fig. 2).

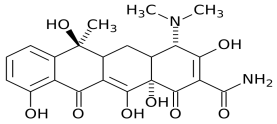

Figure 2: tetracycline

Macrolides have similar mode of action as tetracyclines, namely affecting the metabolism of the bacteria (Sjukvårdsrådgivningen, 2011). The difference is that macrolides bind reversibly to the 50 S subunit of ribosomes, instead of the 30 S ribosomes (Halling-Sørensen, 2001). They are only effective against the gram positive bacteria (Halling-Sørensen, 2001) and are mainly used in treatment of infections caused by mycoplasma, such as pneumonia (Sjukvårdsrådgivningen, 2011). Common active substances are e.g. erythromycin (fig. 3) and azitromycin.

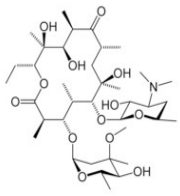

Figure 3: erythromycin

Quinolones inhibit the enzyme DNA gyrase (Halling-Sørensen, 2001), which prevents the division of bacterial cells and therefore stops the bacteria from multiplying (Sjukvårdsrådgivningen, 2011). They are active towards the gram negative bacteria only (Halling-Sørensen, 2001) and are used in more severe cases of e.g. urinary tract infections, gonorrhea, prostate infections and severe intestine infections (Sjukvårdsrådgivningen, 2011). Some common active substances are moxifloxacin, ciprofloxacin (fig. 4), enrofloxacin and ofloxacin.

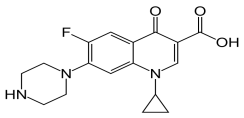

Figure 4: ciprofloxacin

Trimethoprim interferes with folate synthesis by inhibiting the production of the enzyme dihydrofolate reductase (van der Grinten et al., 2010), which is essential for the bacteria to be able to multiply (Sjukvårdsrådgivningen, 2011). It is a broad spectrum antibiotic and is effective on both gram positive and gram negative bacteria (van der Grinten et al., 2010) It is mostly used for treatment of urinary tract infections (Sjukvårdsrådgivningen, 2011). The active substance is also called trimethoprim (fig. 5).

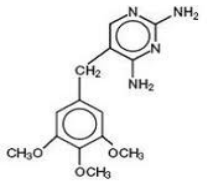

Figure 5: trimethoprim

Sulfonamides inhibit the DNA/RNA synthesis of dihydrofolic acid through the inhibition of the enzymatic step, dihydropterate synthetase (Halling-Sørensen, 2001), which affects the metabolism of the bacteria and thus cause lethal effects (Sjukvårdsrådgivningen, 2011). The drug is useful for both gram positive and gram negative bacteria (Halling-Sørensen, 2001). Sulfonamides are often used together with trimethoprim in treatment of severe urinary tract infections, because it enhances the inhibition (Sjukvårdsrådgivningen, 2011). Active substances in sulfonamides are e.g. sulfamethoxazole (fig.6) and sulfadiazine.

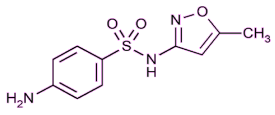

Figure 6: sulfamethoxazole

1.4. The process in sewage treatment plants

STPs can look quite different from one another. They can e.g. vary significantly in which treatment processes they use. In Sweden there are however (according to Lindberg et al 2006), three steps of treatment that the wastewater usually undergo but the order in which they appear can differ from one treatment plant to another. The steps are: a mechanical, where

screening and removal of sand, fat and other solid material occurs; a chemical, including flocculation of phosphorus with ferrous sulphate or ferric chloride; and also a biological step, where the organic matter in the wastewater is degraded by microorganisms. The formed sludge in the three steps are removed from the water, either by clarifiers or filtration, and put in a digestion tank where the organic matter can be degraded (Lindberg et al., 2006). The retention time in the digester has a minimum of 15 days (Naturvårdsverket, 2008). Lastly the amount of water in the sludge is reduced through the process of dewatering (Lindberg et al., 2006). In figure 7, a schematic picture over the processes is given.

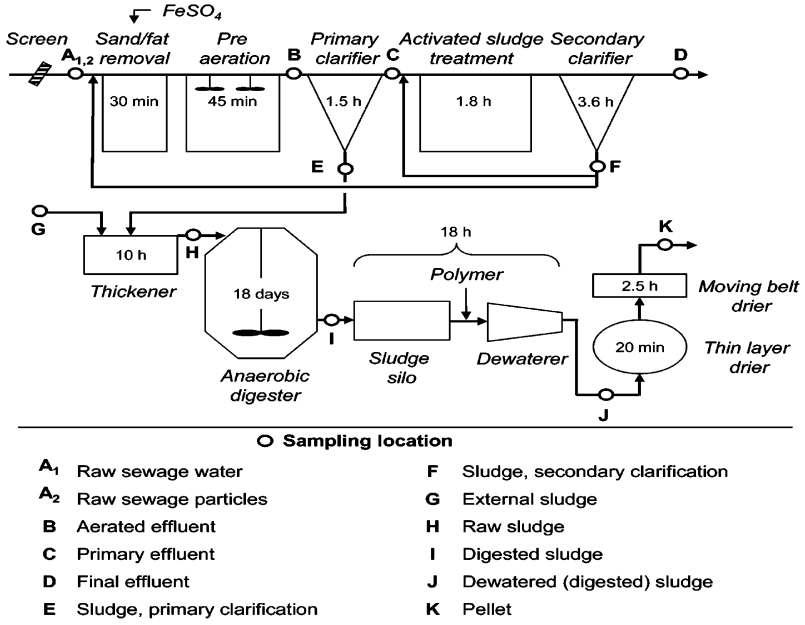

Figure 7: A scheme over the treatment process in a Swedish STP, in Umeå (Lindeberg et al., 2006).

Different removal routes for the compounds in the STPs can be binding and/or adsorption to sludge, biodegradation, hydrolysis, photolysis and vaporization (Eriksen et al., 2009, Kim et al., 2005). Exactly what happens to the antibiotics in the STPs is, however, still rather unclear. Pharmaceuticals are known to be quite difficult to remove and one of the reasons is that they all look rather different from one another, which can bring about that they do not respond to the same treatments. Hence, to find treatments that effectively remove all the pharmaceuticals can be somewhat advanced and costly.

1.5. The process of putting sludge on the field

The regulations concerning the process of spreading biosolids on arable land is given in the Swedish constitutions SNFS 1994:2 and SJVFS 2004:62. There is e.g. information regarding on which fields it is allowed to use biosolids, regulations of the maximum permitted metal content, the permitted level of phosphorus that can be added to the soil and more. The amount of biosolids that can be added to the soil is actually decided by its content of phosphorus (Leander, 2011). The allowed amount, during a five years period, is not allowed to exceed the amount corresponding to 22 kilos of phosphorus per hectare of dispersal area and year (SJVFS 2004:62). This number usually amounts to a dispersal of biosolids between 10-15 tonnes per hectare and year (Leander, 2011). The added biosolids should be mixed with the soil in the agricultural fields to get an optimal contact area and the depth, to which the sludge should be added, varies with the time of year and specific properties of the soil but lies around 5-10 cm (SJVFS 2004:62).

2. Method & Material

2.1. RQ

To be able to estimate the risk of adverse effects on soil ecosystems in agricultural fields, a risk analysis was performed (in accordance to Öberg, 2009) with the assistance of the computer software @Risk 5.7. Calculations with @Risk includes the probability of a risk occurring and it also enables you to use distributions instead of mean values in the calculations. The risk was calculated from equation 1:

$$RQ = PEC/PNEC \quad (1)$$

where RQ is the risk quotient, PEC the predicted environmental concentration and PNEC the predicted no effect concentration. If RQ > 1, a risk on the ecosystem could not be excluded but if RQ ≤ 1, 95 % of the species of the ecosystem were supposed to be without any risk. This means that a risk on 5 % of the species was acceptable. @Risk randomly sampled values from the different distributions for the calculation of RQ. The calculations were also iterated (in this case 10,000 times) to get a relative frequency of the RQ-values. The relative frequency of RQ exceeding 1, presents the probability of a risk occurring.

2.2. PEC

The PEC-value can be derived from equation 2, which in this study has been designed to provide an estimation of the concentration of antibiotics that end up in the sludge in the STPs.

$$PEC = (A \cdot E \cdot R) / V \quad (2)$$

where A is the amount (g) of antibiotics consumed in Sweden in one year, E is excretion rate (%) of the antibiotics from the body, R is the removal rate (%) in the the STPs and V is the total volume of sewage sludge annually produced in Swedish STPs.

2.2.1. Amount

The total amounts of antibiotics annually sold in Sweden (appendix 1) were used to estimate the amount of antibiotics that reach the STPs. The amounts were given in the unit, defined daily dose (DDD) and were then translated into units of gram with help from a DDD index from the WHO (WHO, index). Antibiotics belonging to the same group were summed up and one amount was given for the whole group. A distribution of the total annual amounts sold between the years 2007 and 2010 was made for each of the six groups of antibiotics. It was assumed that all the antibiotics were consumed the same year as they were bought and that nothing was disposed in the garbage. It was also assumed that all the antibiotics passed through the human body before they reached the STPs. Only antibiotics used for human treatment were included in the calculation.

2.2.2. Excretion

Information about the excretion rate was collected from different sources in the literature and fitted into a distribution (appendix 2). Antibiotics from the same group were considered as equals.

2.2.3. Removal

To assess the amount of antibiotics that ended up in sludge, a conservative scenario was used. It was assumed that all of the antibiotics that were removed in the treatment process were adsorbed to sludge. Consequently, no biodegradation, volatilization, hydrolysis or photolysis was assumed to occur. The removal rate of the antibiotics in the STP has been used in a sort of reversed way from usual, when estimating how much that will end up in the effluents. The removal rate has in this case been used to calculate what will end up in the sludge (appendix 3).

Instead of using the removal rate, to estimate how much of the antibiotics that stick to sludge, the partitioning coefficient (K_d) between sludge and water is often used. The K_d is different for every substance and vary depending on the environment. It can be calculated by equation 3 (Halling-Sorensen et al., 2000).

$$K_d = f_{oc} \cdot 0.41 \cdot (K_{ow} / 1 + 10^{pH-pKa}) \quad (3)$$

where f_{oc} is the fraction of carbon in the sludge and K_{ow} is the partitioning between octanol and water. The f_{oc} and the pH vary in different STPs and therefore some kind of mean or a distribution of the values from different STPs would have had to be used. In a review, Tolls (2001) compared the K_d-coefficients for different antibiotics and found that different studies had found a very varying range of values for the same kind of antibiotic. Le-Minh et al. (2010) found that the K_d coefficient varied in different treatment conditions, e.g. in digested or activated sludge. According to Tolls (2001), there are many factors affecting the adsorption potential of antibiotics, which are usually not considered when estimating K_d. For example, tetracyclines were better adsorbed when the cation exchange sites were occupied by Ca^{2+} instead of Na^{+}, the antibiotics seemed to easier adsorb if the surface area of the matter to which they adsorb were larger, and the antibiotics seemed to be able to bond by H-bonding or covalent bonding and not just by ionic bonding etc. This indicates that equation 3 might be inadequate for estimating the partitioning of antibiotics to soil/sludge. In fact, using that method might underestimate the fraction adsorbed. The removal rate (which was used in this study) was, however, estimated from the difference in inflow and outflow of antibiotics in the wastewater and this number could of course also be somewhat incorrect. It was assumed though, that the mentioned factors above would have caused an equal amount of (or higher) uncertainty to the removal variable as using the removal rate did. However, calculating with the K_d-coefficient would perhaps be the best alternative if it was the amount in sludge from a specific STP that was of concern, when the f_{oc} and the pH could rather easily be measured.

2.2.4. Volume

The volume was estimated from the annual volume of sewage sludge that had been produced in the Swedish STPs.

2.3. PNEC

To derive a PNEC a species sensitivity distribution (SSD) was performed according to Posthuma et al. (2002). Toxicity data for the different antibiotic groups were collected from the literature (appendix 4). The aim was to find toxicity data for soil ecosystems but data was mainly found for bacteria and fungus and only a very few for organisms higher up in the trophic levels (earthworms, nematodes and collembolan). To compensate for this, data from

the aquatic environment have been used, including organisms that are assumed to be somewhat comparable. The aquatic organisms constitutes of bacteria, primary producers (algae and plants) and different grazers (zooplankton). The toxicity data consist of different EC-values (effect concentration), MICs (minimum inhibition concentration) and NOECs (no effect concentrations). When possible, the NOEC has been used, since it is assumed to be the more conservative value. Effects on the few soil organisms that were found have been discussed in a chapter of its own after the risk analysis.

2.4. Fate in soil & Resistance

Facts about how the antibiotics behave in soil and about the resistance have been gathered from different sources in the literature.

3. Results

3.1. Risk analysis

The calculated PECs and PNECs, for the different antibiotic groups, can be seen in table 1. The PEC-values for the penicillins, tetracyclines, macrolides and quinolones were around three orders of magnitude higher than the PNEC-values. For the trimethoprim and sulfonamides the number was significantly lower, with the PECs and the PNECs being around the same magnitude or somewhat higher. Specific data for the PEC variables; amount, excretion rate and removal rate, are found in appendix 1, 2 and 3 respectively. The volume of sewage sludge annually produced in Swedish STPs amounted to about $1 \cdot 10^9$ kilos (Naturvårdsverket, 2011). It was assumed that water and sludge have similar density and therefore the volume was approximated to $1 \cdot 10^9$ litres. The specific toxicity data for the PNECs are found in appendix 4.

Table 1: Mean PEC- and PNEC-values, with standard deviations (std) and the whole range of sampled values, for the different antibiotic groups, after calculation with @Risk. The PECs are the predicted concentration in sludge from Swedish sewage treatment plants and the PNECs are the concentration where soil organisms are assumed to be affected by the antibiotics.

Name	PEC (ug/L)	std	range	PNEC (ug/L)	std	range
penicillins	31000	6200	14000 - 47000	41	31	0.0023 - 190
tetracyclines	2700	340	1800 - 3600	5.5	2.8	1.1 - 15
macrolides	1200	520	230 - 3000	6.0	3.9	0.33 - 22
quinolones	1700	350	720 - 2900	3.9	1.9	0.87 - 11
trimethoprim	95	61	0.00029 - 340	46	35	0.0033 - 210
sulfonamides	490	330	0.060 - 1800	5.1	2.5	1.1 - 14

The risk analysis showed a very high risk of all of the compounds giving negative effects on the soil ecosystem. The penicillins, tetracyclines, macrolides and quinolones constituted the highest risks. Out of 10,000 iterations, all of the RQ-values were found above one (fig. 3, 4, 5 and 6). The trimethoprim and sulfonamide drugs showed lower but still high risks with 74.4 % and 99.4 % respectively of the RQs above one (fig. 7 and 8).

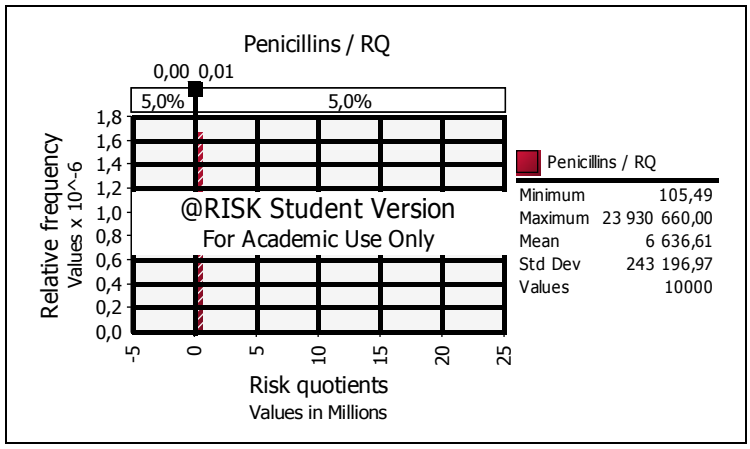

Figure 3: The risk of adverse effects on soil ecosystems from penicillin in biosolids. A relative frequency of risk quotients (RQ) for the penicillin drugs is given. Out of 10,000 iterations, all of the RQ-values were found above one.

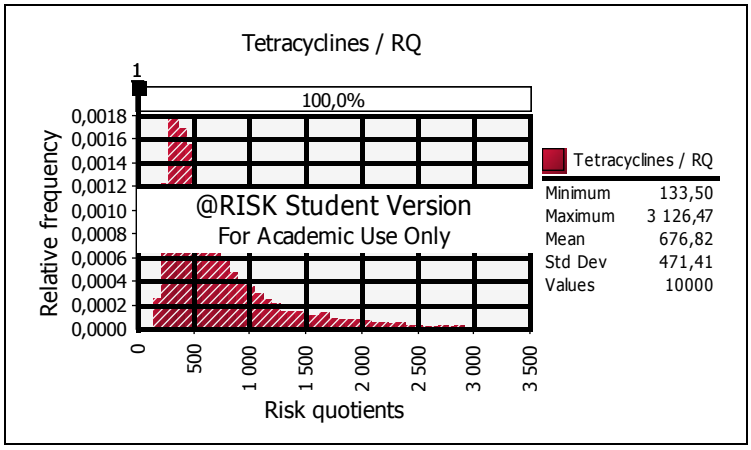

Figure 4: The risk of adverse effects on soil ecosystems from tetracyclines in biosolids. A relative frequency of risk quotients (RQ) for the tetracycline drugs is given. Out of 10,000 iterations, all of the RQ-values were found above one.

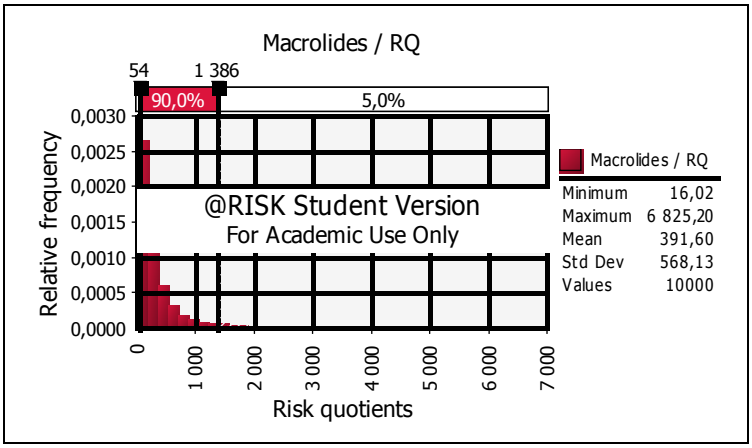

Figure 5: The risk of adverse effects on soil ecosystems from macrolides in biosolids. A relative frequency of risk quotients (RQ) for the macrolide drugs is given. Out of 10,000 iterations, all of the RQ-values were found above one.

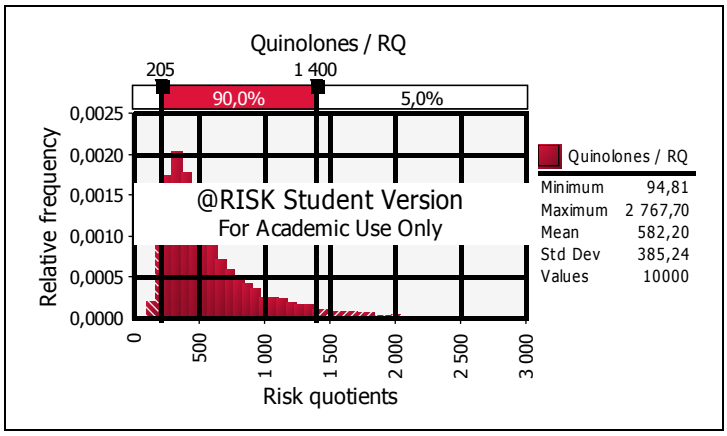

Figure 6: The risk of adverse effects on soil ecosystems from quinolones in biosolids. A relative frequency of risk quotients (RQ) for the quinolone drugs is given. Out of 10,000 iterations, all of the RQ-values were found above one.

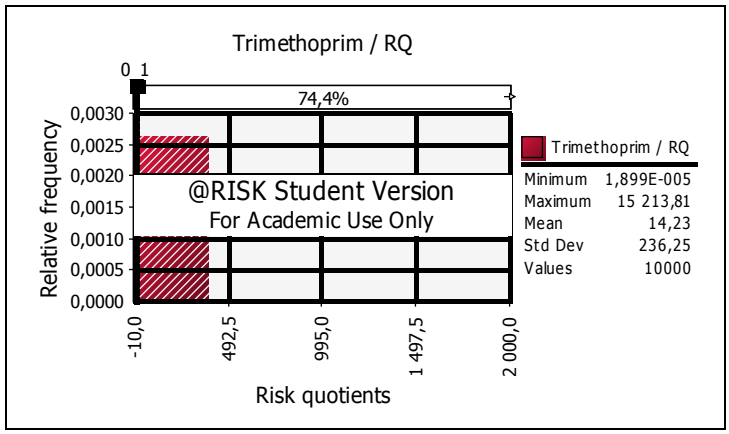

Figure 7: The risk of adverse effects on soil ecosystems from trimethoprim in biosolids. A relative frequency of risk quotients (RQ) for the trimethoprim drugs is given. Out of 10,000 iterations, 74.4 % of the RQ-values were found above one. Note that the x-axis is truncated.

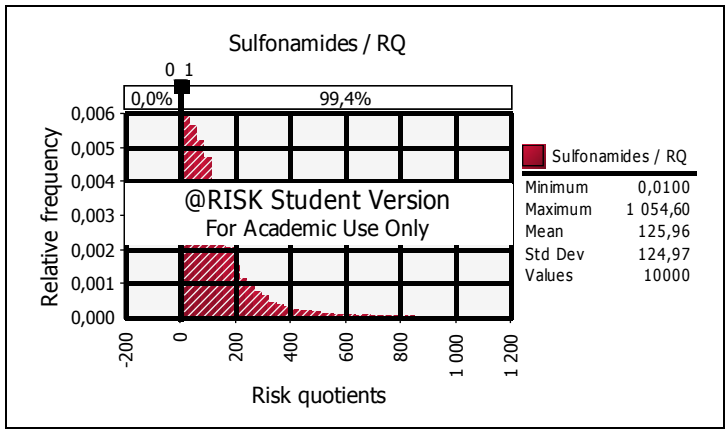

Figure 8: The risk of adverse effects on soil ecosystems from sulfonamides in biosolids. A relative frequency of risk quotients (RQ) for the sulfonamide drugs is given. Out of 10,000 iterations, 99.4 % of the RQ-values were found above one.

3.2. Effects on soil organisms

Most of the toxicity data in this study have been taken from the aquatic environment. The reason for that was the problem of finding data for soil organisms, since such studies were found to be very scarce in the literature. There are some for microbes but studies on organisms higher up in the food chain are lacking distinctively. Only a very small number have been made. The following are some examples of toxicity studies on soil organisms:

Halling-Sørensen (2001), made an experiment with the nitrifying bacteria, *Nitrosomonas europaea,* to test the inhibition of growth and nitrification when exposed to different antibiotics. It was expected that, since it was a gram negative bacteria, it should be affected by the broad spectrum antibiotics and by the ones that are active against the gram negative bacteria. The results showed that the tetracyclines inhibited both growth and nitrification, with an EC_{50}-value at 0.64 mg/L for chlortetracycline. It was also found that some antibiotics (sulfadiazine and tylosin) stimulated the nitrification.

Bauger et al. (2000) tested the toxicity on three species of soil fauna; collembolan, springtail and earthworms towards the antibiotics oxytetracycline and tylosin. According to their results, from tests on the endpoints survival, fertility, growth and cocoon hatchability, the organisms seemed to tolerate rather high levels of the antibiotics. The EC_{10}-values ranged from 134 to more than 5000 mg/kg. In another rapport by Kumar et al. (2005), toxicity data for oxytetracyclines, sarafloxacin and tylosin were gathered for their toxicity on earthworms and collembolan (data originally from Boxall et al., 2004), which showed similar results with EC_{50}-values from 900 to above 5000 mg/kg.

Plants have also been found to be affected by some of the antibiotics. Sulfadimethoxine for example, significantly suppresses the growth of roots and leaves of the barley *Hordeum distichum* at a concentration of 300 mg/L (Migliorie et al., 1996). The plant bioaccumulated the drug and the higher the accumulation rate the more toxic it was found to get. The toxicity of enrofloxacin was tested on the crop plants *Cucumis sativus, Lactuca sativa, Phaseolus vulgaris* and *Raphanus sativushas* by Migliorie et al. (2003). In lower concentrations (50-100 µg/L) the drug mainly seemed to stimulate the growth of roots, hypocotyl, cotyledons and leaves but in higher concentration (100-5000 µg/L) it gave rise to toxic effects by inhibiting the growth.

3.3. Fate of the antibiotics in the environment

The persistence of antibiotic drugs in soil depends partially on the intrinsically properties of the antibiotics (table. 2) and also on the soil properties and the weather conditions of the environment (Kumar et al., 2005). The properties of the antibiotics can be binding, and adsorption to soil, photostability, biodegradation and water solubility (Kumar et al., 2005). A low water solubility and a high log K_{ow} usually means that the compound have a high potential for adsorbing to soil. Also important is their ability to form ions, which, depending on the charge, are able to bond to the soil. The adsorption was found to be stronger if the clay content of the soil is high (Kumar et al., 2005) or if the organic matter content is high (Golet et al., 2003).

The biodegradation depends on several factors, such as temperature, redox-potential and the capability of the microorganisms to transform or degrade the antibiotics (Eriksen et al., 2009) Abiotic degradation, in form of hydrolysis and photooxidation, does not play a significant role in soil (Eriksen et al., 2009). In a field test carried out by Golet et al., (2002), quinolones were

found to persist months after sludge had been applied to the field. Tetracyclines were also found to be rather persistent. An OECD test showed that only 1% had broken down after 28 days (FASS, 2011)

Table 2: Physiochemical properties of six groups of antibiotics (Thiele-Brun et al., 2003, Pérez et al., 2005).

Compounds	molar mass (g/mol)	water solubility (mg/l)	log K_{ow}	pKa	Henry's constant
Penicillins	334.4-470.3	22-10100	0.9-2.9	2.7	2.5E-19 - 1.2E-12
Tetracyclines	444.5-527.6	230-52000	(-)1.3-0.05	3.3	3.3/7.7/9.3
Macrolides	687.9-916.1	0.45-15	1.6-3.1	7.7-8.9	7.8E-36 - 2.0E-26
Quinolones	229.5-417.6	3.2-17790	(-)1.0- 1.6	8.6	5.2E-17 - 3.2E-8
Trimethoprim	290.32	500	0.91	6.6	-
Sulfonamides	172.2-300.3	7.5-1500	(-)0.1-1.7	2-3/ 4.5-10.6	1.3E-12 - 1.8E-8

3.4. The issue on resistance

Resistance towards antibiotics among bacteria can evolve either through selective pressure on the bacterial strains, mutation or through the acquirement of new DNA from other resistant bacteria (Tenover FC, 2006). The selective pressure is caused by a repeated exposure of the antibiotics, which might lead to the emergence of new resistant strains. A mutation could e.g. be a change in the binding site of a target protein, making the antibacterial agent unable to connect, which in turn prevents the reaction from occurring. Acquiring the resistance from other bacteria can occur in three different ways; through conjugation, transduction and transformation. For gram negative bacteria the conjugation occurs through the transfer of genes that are carried in plasmids, via two adjacent bacteria, which are connected via a protein-like extension. For gram positive bacteria the exchange occurs between two mating pairs. Transduction means that the genes are transferred between bacteria, like a bacterial virus. Lastly, transformation means the process of obtaining segments of DNA that have been released into the environment by other bacteria, after cell lysis.

In a review by Kim and Aga (2007) it was claimed that the conditions in STPs are fairly suitable for the spreading of resistance among bacteria. An example brought up (performed by Mach and Grimes, 1982), showed a higher resistance transfer between bacteria in an in-situ experiment in primary and secondary clarifier effluents compared to in a laboratory experiment, which was an indication of a more favourable transfer in the STPs. Another test showed a significant increase in resistance towards different antibiotics among the strains of *Enterobacteriacea* and *Aeromonas* downstream a STP effluent in Spain (Goni-urriza et al., 2000).

Resistant bacteria are found in both untreated and treated sewage sludge but the level is lower in treated (Eriksen et al., 2009). When biosolids are put on agricultural fields the antibiotics can still occur in an active form (Chander et al., 2005). This could create a selective pressure on the bacteria for the development of antibiotic resistance (Chander et al., 2005). However, according to Sengeløv et al. (2003) the contribution of antibiotic is often too small to give lasting effects. In a field test, lasting for 8 months, they applied pig manure onto farm lands to study how the resistance among the bacteria developed. At first, they could see an increase of the resistance against the tested antibiotics; tetracycline, macrolides and streptomycin. Although, for the two latter there were only minor differences observed, compared with the starting point. Tetracycline resistance was shown to be very influenced by the addition of manure to the soil and levels increased significantly. However, the level of resistance declined rather quickly with time back to the control level. So, if no additional manure (or presumably biosolids) was to be added, the resistance among the bacteria would likely be lost due to loss

in competitive advantage. The persistence and mobility of the antibiotics are also of importance for the development of antibacterial resistance. If the persistence is high and the mobility low, the likelihood of developing resistance will be greater (Eriksen et al., 2009).

It has been shown that antibiotics can be taken up by crop plants (Migliore et al., 2003). If the bacteria on the crops have developed antibacterial resistance these strains could be transferred to humans via the ingestion of the crops, which in turn could promote the resistance of bacteria in humans (McClellan and Halden, 2010). If resistance among pathogenic bacteria is able to develop it can spread around the world in a rather short time (Naturvårdsverket, 2008).

4. Discussion

According to the results the soil environment does seem to be at risk of being affected by antibiotics in biosolids. Especially penicillins, tetracyclines, macrolides and quinolones were found to have a very high probability of reaching concentrations which would give rise to negative effects on the soil environment, since all RQ-values were found above one. The other two groups, trimethoprim and sulfonamides, had somewhat lower RQ-values but were despite that also found to constitute high risks.

These findings were, however, somewhat contradictive to another study, performed on the same matter by Eriksen et al. (2009). They came to the conclusion that the antibiotics in biosolids would not constitute any harm on the soil environment. This was, however, a Norwegian study and the results are therefore not completely comparable, since they have other numbers on the consumption of antibiotics and on the produced volume of sewage sludge. Measured amounts in sludge from Swedish STPs have found to be fairly high for some of the antibiotics. In a study of five Swedish STPs by Lindberg et al. (2005), the amount of antibiotics in the produced sewage sludge were measured and concentrations of quinolones were found as high as 4.8 mg/kg d.w and of tetracyclines as high as 1.5 mg/kg d.w. A Swiss and an U.S study also found mostly quinolones and tetracyclines and in similar concentrations as the Swedish study (Golet et al., 2002, McClellan and Halden, 2010). McClellan and Halden (2010) did also find macrolides in the sludge, in concentrations up to 0.8 mg/kg d.w, and trimethoprim, in concentrations around 0.026 mg/kg d.w. However, none of the studies found any traces of penicillins or sulfonamides. These findings seem to support the results of the present study in some degree and the results by Eriksen et al. (2009) in some. The risk of penicillins and sulfonamides does e.g. seem to be rather exaggerated, which might be due to the exclusion of degradation in the model. However, the measured values of the other antibiotics are, according to the PNEC, high enough to give rise to adverse effects on the soil environment. An important factor for the risk was however also, the persistence of the antibiotics in the soil environment. Tetracyclines and quinolones were the only ones that have shown to be persistent. A factor that would assumingly also decrease the risks is the dilution effect, when biosolids are added and mixed with agricultural soils, which was considered in the Norwegian study but not in this. On the other hand, mixture toxicity (which will be discussed more later on) has not been considered in either of the studies and that factor might instead increase the risks.

Eriksen et al. (2009) also made the judgement that antibiotic resistance was not promoted in soil after the appliance of biosolids, with a possible exception of resistance towards quinolones. Since both the level and the exposure time matters (Sengeløv et al. 2003) this study supports the assumption that the quinolones, but possibly also the tetracyclines, are at greatest risk of developing resistant bacterial strains, as they were the most persistent ones in

the soil environment. Since antibiotic resistance is a very serious matter it is important that no rash conclusions are drawn.

The findings of the risks are based on the correctness of the variables in the two equations that were used for the risk estimation. However, there are some uncertainties with the variables that are discussed in the part below.

4.1. PEC

4.1.1. Amount

In the calculation of the amount it was assumed that all the antibiotics were consumed in the same year as they were bought and that nothing was thrown away in the trash. It was also assumed that all the antibiotics had passed through the human body before they reached the STPs. This might have overestimated the amount a little but perhaps not that much. Antibiotics are after all prescribed in courses, for treatment of ongoing diseases and the course is supposed to be eaten directly and to the finish to be sure of recuperation. That no veterinarian antibacterial drugs were included might instead have underestimated the amount since it is possible that some of them reach the STPs, e.g if excretion from animals, whom have been treated with antibiotics or have had it fed into their diet, reach the surface water gullies. A source of error could also be if there was incorrectness in the statistics of the sell of antibiotics.

4.1.2. Excretion

The literature gave very varied numbers of the excretion rates, which made that parameter rather uncertain. This affects the certainty of the whole risk analysis and more focus should therefore be put on a more thorough examination of how the antibiotics pass the body. This includes also the examination of in which form, unchanged or as metabolites, the antibiotics are excreted. It seems like most of the data of the excretion rate are of antibiotics in an unchanged form, which could be because it is easier to measure since you know what to look for. Also, the metabolites are often less active than the original compound (Halling-Sørensen et al., 2003) and could therefore be thought of as not as important to keep track on. However, studies have shown that, in the STPs, the metabolites can be retransformed into its originally active form. This is shown when the STPs appear to have a negative removal of the compounds, making it look like more antibiotics are leaving the STP than entering. A study by Göbel et al. (2005) found evidence for, that the sulfonamide metabolite N4-acetyl-sulfamethoxazole, which was frequently present in the influent, had been retransformed to its original compound in the STP. Hence, neglecting the metabolites can lead to an underestimation of the amount of active antibiotics in the STPs.

4.1.3. Removal

When it comes to the removal rate, the same problem as with the excretion rate arose, with a lot of different number presented in the literature. This is probably mostly due to that the STPs have varied abilities of removing the antibiotics and that the data is taken from studies from different STPs. The differences in removal efficiency have to do with which treatment processes that are available at the plant and with the residence time of the sewage at the STPs (Gulkowska et al., 2008). It has also been suggested that the temperature of the raw sewage water might affect the proportion of antibiotics removed, by higher adsorption potential with increasing temperature (Lindberg et al., 2006).

To assume that all of the antibiotics that are removed will end up in sludge are in reality not very likely but it was believed to be a conservative estimation of the amount adsorbed to sludge. The assumption seems to be more accurate for some antibiotics than others. The quinolones and tetracyclines are e.g. found to be significantly removed by sorption to sludge (Lindberg et al., 2006, Batt et al., 2007, Kim et al., 2005, Golet et al., 2003, McClellan and Halden, 2010). Both of them are rather water soluble and have a fairly low log K_{ow} (Kim et al., 2005) but the reason for the adsorption was mainly that they are found to react with ions, such as magnesium, calcium and ferric iron, which form solids that can accumulate in sludge (McClellan and Halden, 2010, Hirsch et al., 1999, Turiel et al., 2006). Lindberg et al., (2005) also found that biodegradation of quinolones, in a study of five STPs in Sweden, were negligible, which supports the findings that adsorption is the main removal route. However, the same study did find that tetracyclines might undergo some degradation.

Macrolides are fairly hydrophobic (as can be seen in fig. 2) and have a rather low biodegradability (Ericson, 2007) and are therefore according to McClellan and Halden (2010) likely to at a significant extent end up in the sludge. Azithromycin also has cationic properties, which means that it is likely to bind to soil (Ericson, 2007). However, Göbel et al., (2005) found that the sorption of macrolides to activated sludge were low. The proportion adsorbed might therefore be overestimated. The fate of these antibiotics in the STPs needs to be further investigated.

Many authors (Pérez et al., 2005, Lindberg et al., 2006, Batt et al., 2007, Li and Zhang, 2010) have found that sulfonamides and trimethoprim are poorly bound to sludge due to their hydrophilic characteristics. An US study, which measured the amount of trimethoprim in sludge, did however find a considerable amount of the antibiotic at a concentration of 0.026 mg/kg d.w (McClellan and Halden, 2010). This, on the contrary, indicates that trimethoprim is able to bind to sludge. Another reason for the poor adsorption for sulfonamides were, according to Pérez et al. (2005), that the common sulfonamide compound, sulfamethoxazole, mainly exist in its anionic form in the STPs. Since the surface layer of the sludge also is anionic (Albertson, 1991) it is not likely that any ionic bonding occurs, in a significant amount, between sulfamethoxazole and the sludge.

Penicillins were found to be fairly unstable due to their β-lactam ring, which can easily be broken by the bacterial enzyme, β-lactamases, or by chemical hydrolysis (Cha et al., 2006). Hirsch et al. (1999) found that amoxicillin and ampicillin were eliminated already in an early stage at the STP. This indicates that a substantial amount of what is being removed are actually degraded and not adsorbed to sludge.

The choice of neglecting volatilization as a removal step in STPs does seem to have support from different sources in the literature, claiming that the vapour pressure of the compounds should be too low for any vaporization to occur (Halling-Sørensen et al., 2000, Pérez et al., 2005, Batt et al., 2007).

The amount of antibiotics adsorbed to sludge seems, regarding what has been brought up, to be somewhat overestimated, which has to do with the negligence of degradation. However, it was a consciously made choice, since it was considered to be better to overestimate than to underestimate the adsorbed amount.

4.1.4. Volume

The volume of sewage sludge used in the calculation was taken from one source and did only include an approximate number. No variation of the production was given and consequently no distribution could be made. Unfortunately, no more data of the total amount of sewage sludge in Sweden was found. More exact data would surely have given a higher certainty to the calculation.

4.2. PNEC

The PNEC-values were found to be sensitive enough for many of the organisms to be affected by the levels of antibiotics in biosolids. The penicillin group showed the highest PNEC-value, which gave rise to a very high risk on the ecosystem. However, the spread in toxicity for this group was very high, as they also exhibited the lowest toxicity values. Some organisms, like the blue-green alga, were found to be very sensitive to the penicillin, amoxicillin, exhibiting a NOEC at as low a concentration as 0.78 µg/L (Andreozzi et al., 2004). Other organisms, like the zooplankton *D. magna* and the green alga *S. capricornutum*, showed NOECs as high as 300 mg/L (Halling-Sørensen et al., 2000), suggesting that they were rather insensitive towards penicillin.

The few data found on soil organisms higher up in the tropic levels (earthworm, collembolan and nematode) were shown to be rather insensitive to antibiotics. However, the organisms were only tested for some substances of tetracyclines, macrolides and quinolones and it is not certain that they would respond equally to other types of antibiotics. If the response would be similar, then these organisms are probably more likely to be affected indirectly by a disruption in the ecosystem, caused by negative effects on microbes or plants.

There is a possibility that the PNEC-values are somewhat underestimated since no mixture toxicity has been considered. Backhaus et al. (1999) tested the toxicity of several quinolone antibiotics and found that mixture toxicity has to be considered to not underestimate the risks. Additionally, trimethoprim and sulfonamides have shown to be synergistic when mixed together (Eguchi et al., 2004, Sjukvårdsrådgivningen, 2011). Another study by Yang et al. (2008) showed that mixtures of sulfamethazine and norfloxacin as well as of chlortetracycline and norfloxacin gave slightly synergistic effects. There has also been a study showing that copper and oxytetracycline together enhanced the toxicity on soil microbial community function (Kong et al., 2006) A Norwegian study by Eriksen et al. (2009) has, in addition, found that the concentration of copper in sludge amended soils would reach levels above the permitted maximum concentration after some year of application, which could likely happen in Sweden as well.

On the other hand, it has also been shown that antibiotics can have positive effects on organisms. Stoichev et al. (2010) showed that the degradation products of minocycline can have positive effects on the growth of *Microcystis aeruginosa*, because they constitute a food source. Halling-Sørensen (2001), as was mentioned earlier, found that tylosin and sulfadiazine stimulated the nitrification of the bacteria *Nitrosomonas europaea*. This could therefore also be a possible fate of the antibiotics if they do not occur at toxic levels.

To get a more accurate PNEC-value, toxicity data should be used from studies that have actually tested the response of the organisms of concern. More toxicity tests are therefore needed on soil organisms.

5. Conclusion

The risks for adverse effects on the soil environment from the use of biosolids, regarding its content of antibiotics, were according to the risk assessments very high. The greatest risk was found for the penicillins but it was probably greatly overestimated since a significant amount was believed to degrade in the STPs. Hence, the risk of tetracyclines and quinolones seem to be of greatest concern, since they were found to at a large extent bind to sludge and also persist in the environment. This might also be applied to the macrolides and trimethoprim but information about their behaviour in sludge/soil was too contradictive to make any certain assumptions. Sulfonamides were thought to have a very low adsorption potential to sludge, which reasonably would infer that what was being removed of those compounds was mainly degraded. This should lower the risk of the sulfonamides. However, as been discussed, more factors need to be considered to get an adequate picture over the risks. This study only aimed to make an initial estimation of the risks and to put focus on the areas that needs further investigation to be able to make more reliable risk analyses on the subject.

When it comes to the issue of resistance it could not be concluded from this study that spreading of biosolids on agricultural fields would not promote the development of resistance among bacteria. It is therefore important that the issue on resistance is further investigated

This study only considered antibiotics but they are not the only compounds that might constitute a risk. There are many emerging contaminants that reach the STPs, which might also stick to sludge. To be able to assess the risk of spreading biosolids on agricultural fields, all those substances need to be considered.

Hence, with present knowledge the usage of biosolids on agricultural fields does seem to have a fairly high probability of generating negative effects, at least for the soil environment but possibly also for humans, if antibiotic resistance should appear to be promoted. Therefore, until adequate information about the risks is presented, the spreading of biosolids on agricultural fields should preferably be sparse.

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Appendix 1.

Table 3: The amount DDD of six groups of antibiotics sold in the years 2007, 2008, 2009 and 2010. The DDD's are translated to amounts in gram. (Apoteket 2009, Apoteket 2010, WHO index)

				DDD-amount				Amount (g)			
ATC code	**Name**	**DDD-value**	**U**	**2007**	**2008**	**2009**	**2010**	**2007**	**2008**	**2009**	**2010**
	Pencillins										
J01CA01	ampicillin	2	g	59582	63195	63242	60444	119164	126390	126484	120888
J01CA02	pivampicillin	1.05	g	67				70.35			
J01CA04	amoxicillin	1	g	3799017	3816039	3544232	3638829	3799017	3816039	3544232	3638829
J01CA08	pivmecillinam	0.6	g	1763937	2014343	2053259	2092914	1058362	1208606	1231955	1255748
J01CA11	mecillinam	1.2	g	311	2	7	0	373.2	2.4	8.4	0
J01CE01	benzylpenicillin	3.6	g	148032	197314	241092	234592	532915.2	710330.4	867931.2	844531.2
J01CE02	phenoxymethylpenicillin	2	g	14841273	14823503	13928341	13949487	29682546	29647006	27856682	27898974
J01CF01	dicloxacillin	2	g	38950	22056	612	0	77900	44112	1224	0
J01CF02	cloxacillin	2	g	407138	467458	528257	558974	814276	934916	1056514	1117948
J01CF05	flucloxacillin	2	g	4511340	5295825	5293512	5299687	9022680	10591650	10587024	10599374
J01CR02	amoxicillin and enzyme inhibitor	3	g	878901	904895	864399	882782	2636703	2714685	2593197	2648346
J01CR05	piperacillin and enzyme inhibitor	14	g	92567	127741	157125	184642	1295938	1788374	2199750	2584988
	Total							**49918846**	**52487006**	**50929401**	**51592409**
	Tetracyclines										
J01AA02	doxycycline	0.1	g	7371789	6729163	5938546	5972167	737178.9	672916.3	593854.6	597216.7
J01AA04	lymecycline	0.6	g	4081477	4290274	4458920	4672970	2448886	2574164	2675352	2803782
J01AA06	oxytetracycline	1	g	187183	146768	133942	123778	187183	146768	133942	123778
J01AA07	tetracycline	1	g	811396	769009	691904	629513	811396	769009	691904	629513
J01AA12	tigecycline	0.1	g	2319	2812	3293	4558	231.9	281.2	329.3	455.8
	Total							**4184876**	**4163139**	**4095382**	**4154746**
	Macrolides										
J01FA01	erythromycin	2	g	1563100	1296687	1085310	693525	3126200	2593374	2170620	1387050
J01FA02	spiramycin	3	g		50				150		
J01FA06	roxithromycin	0.3	g	43773	35266	26056	22998	13131.9	10579.8	7816.8	6899.4
J01FA09	clarithromycin	1	g	227761	225397	216352	206125	227761	225397	216352	206125
J01FA10	azithromycin	0.5	g	226866	226501	224184	231693	113433	113250.5	112092	115846.5
J01FA15	telithromycin	0.8	g	560	315	135	150	448	252	108	120
	Total							**3480974**	**2943003**	**2506989**	**1716041**
	Quinolones										
J01MA01	ofloxacin	0.4	g	19146	17160	17349	15023	7658.4	6864	6939.6	6009.2
J01MA02	ciprofloxacin	1	g	2919374	2873296	2901596	2930361	2919374	2873296	2901596	2930361
J01MA06	norfloxacin	0.8	g	633384	372376	189977	137179	506707.2	297900.8	151981.6	109743.2
J01MA12	levofloxacin	0.5	g	63286	63179	68689	69258	31643	31589.5	34344.5	34629
J01MA14	moxifloxacin	0.4	g	77912	65073	60602	64028	31164.8	26029.2	24240.8	25611.2
	Total							**3496547**	**3235680**	**3119103**	**3106354**
	Trimethoprim										
J01EA01	trimethoprim	0,4	g	1631059	1386683	1106143	996319	652423.6	554673.2	442457.2	398527.6
	Total							**652423.6**	**554673.2**	**442457.2**	**398527.6**
	Sulfonamides										
J01EC01	sulfamethoxazole	2	g	795101	860328	914953	953933	1590202	1720656	1829906	1907866
J01EC02	sulfadiazine	0.6	g	9050	6550	5675	6575	5430	3930	3405	3945
	Total							**1595632**	**1724586**	**1833311**	**1911811**

Appendix 2.

Table 4: The range of excretion rates, from the human body, for the six antibiotics. The numbers are collected from different sources in the litterature.

Name	Excretion rate %	Ref.
penicillin	30-90	Hirsch et al. 1999, Al-Ahmad et al. 1999
tetracyclines	60-90	Hirsch et al. 1999, Isidori et al. 2005
quinolones	30-85	Volmer et al. 1997, Lindberg et al. 2005, Al-Ahmad et al. 1999, Kümmerer et al 2000, Isidori et al. 2005
macrolides	60-90	Hirsch et al. 1999, Isidori et al. 2005
trimethoprim	50-70	Hirsch et al. 1999, Lindberg et al. 2005
sulfonamides	15-90	Hirsch et al. 1999, Lindberg et al. 2005, Al-Ahmad et al. 1999, Isidori et al. 2005

Appendix 3.

Table 5: The range of removal rates for the six antibiotics, collected from studies of different sewage treatment plants.

Name	Removal rate %	Ref.
penicillin	90-100	Li and Zhang 2010, Watkinson et al. 2007
tetracyclines	70-98	Li and Zhang 2010, Gulkowska et al. 2008, Lindberg et al. 2005
macrolides	19-100	Gulkowska et al. 2008, Carballa et al. 2007,
quinolones	78-93	Li and Zhang 2010, Golet et al. 2003, Watkinson et al. 2007, Gulkowska et al. 2008, Lindberg et al. 2006, Lindberg et al. 2005
trimethoprim	0-62	Lindberg et al. 2005, Gulkowska et al. 2008, Lindberg et al. 2006
sulfonamides	0-100	Li and Zhang 2010, Carballa et al. 2007, Watkinson et al 2007, Le-minh et al. 2010, Lindberg et al. 2005

Appendix 4.

Table 6: Toxicity data from different soil living and aquatic organisms exposed to penicillin drugs.

Penicillin					
Name	**Species**	**Test**	**Duration**	**Conc. (mg/l)**	**Ref.**
pencillin G	sewage sludge bacteria (pour plate)	EC50 (growth)	48 h	10	Halling-Sorensen 2001
mecillinam	sewage sludge bacteria	EC50 (OECD test)	OECD standard	63	Halling-Sorensen et al. 2000
ampicillin	*V. fischeri* (luminescent bacteria)	EC10 (bioluminescence)	24 h	90	Backhaus et Grimme 1999
amoxicillin	M. aeruginosa (cyanobacteria)	EC50 (growth)	7 d	0.0037	Holten Lützhoft et al. 1999
mecillinam	*M. aeruginosa* (cyanobacteria)	EC50 (OECD test)	OECD standard	0.060	Halling-Sorensen et al. 2000
pencillin G	*S. capricornutum* (green alga)	NOEC (growth)	3 d	100	Halling-Sorensen 2000
amoxicillin	S. capricornutum (green alga)	NOEC (growth)	3 d	250	Holten Lützhoft et al. 1999
mecillinam	*S. capricornutum* (green alga)	NOEC (OECD test)	OECD standard	300	Halling-Sorensen et al. 2000
amoxicillin	*S. leopoliensis* (blue-green alga)	NOEC (growth)	96 h	0.00078	Andreozzi et al. 2004
mecillinam	*D. magna* (zooplankton)	NOEC (OECD test)	48 h	300	Halling-Sorensen et al. 2000

Table 7: Toxicity data from different soil living and aquatic organisms exposed to tetracycline drugs.

Tetracyclines					
Name	**Species**	**Test**	**Duration**	**Conc. (mg/l)**	**Ref.**
tetracycline	sewage sludge bacteria (pour plate)	EC50 (growth)	48 h	0.32	Halling-Sorensen 2001
oxytetracycline	sewage sludge bacteria	EC50 (growth)	48 h	0.12	Halling-Sorensen et al. 2003
tetracycline	*B. megaterium* (bacteria)	MIC (growth)	10 d	0.0050	Wei et al. 2009
tetracycline	*V. fischeri* (luminescent bacteria)	EC10 (bioluminescence)	24 h	0.0046	Backhaus et Grimme 1999
oxytetracycline	*V. fischeri* (luminescent bacteria)	EC25 (bioluminescence)	30 min	65	Isidori et al. 2005
tetracycline	*R. radiobacter* (bacteria)	MIC (growth)	14 d	0.12	Popowska et al. 2010
tetracycline	*A. salmonicida* (bacteria)	MIC (growth)	14 d	0.040	Popowska et al. 2010
tetracycline	*B. vesicularis* (bacteria)	MIC (growth)	14 d	0.12	Popowska et al. 2010
tetracycline	*B. cepacia (*bacteria)	MIC (growth)	14 d	0.056	Popowska et al. 2010
tetracycline	*S. maltophilia* (bacteria)	MIC (growth)	14 d	0.18	Popowska et al. 2010
oxytetracycline	*M. aeruginosa* (cyanobacteria)	EC50 (photosyntetic yield)	24 h	5.4	van der Grinten et al. 2010
chlortetracycline	*M. aeruginosa* (cyanobacteria)	EC50 (growth)	7 d	0.050	Halling-Sorensen 2000
tetracycline	*D. carota* (fungus)	LOEC (root lenght)	28 d	1.0	Hillis et al. 2008
tetracycline	T. intraradices (fungus)	LOEC (hyphal lenght)	28 d	0.30	Hillis et al. 2008
oxytetracycline	*P. subcapitata* (green algea)	EC50 (growth)	72 h	0.17	Isidori et al. 2005
chlortetracycline	*S. capricornutum* (green alga)	EC50 (growth)	3 d	3.1	Halling-Sorensen 2000
oxytetracycline	*D. magna* (zooplankton)	EC50 (immobilization)	24 h	23	Isidori et al. 2005
oxytetracycline	*T. platyurus* (crustacean anostraca)	LC50 (survival)	24 h	25	Isidori et al. 2005
oxytetracycline	*C. dubia* (crustacean cladocera)	EC50 (population growth)	7 d	0.18	Isidori et al. 2005
oxytetracycline	*B. calyciflorus* (rotifer)	EC50 (population growth)	48 h	1.9	Isidori et al. 2005

Table 8: Toxicity data from different soil living and aquatic organisms exposed to macrolide drugs.

Macrolides					
Name	**Species**	**Test**	**Duration**	**Conc. (mg/l)**	**Ref.**
tylosin	sewage sludge bacteria (pour plate)	EC50 (growth)	48 h	6.1	Halling-Sorensen 2001
tylosin	bacterial plate S (senitive to sulphonamides)	EC50 (growth)	24 h	1.1	van der Grinten et al. 2010
tylosin	bacterial plate M (senitive to macrolides)	EC50 (growth)	24 h	0.57	van der Grinten et al. 2010
tylosin	*M. aeruginosa* (cyanobacteria)	EC50 (photosyntetic yield)	24 h	0.29	van der Grinten et al. 2010
tylosin	*D. carota* (fungus)	EC10 (root lenght)	28 d	0.042	Hillis et al. 2008
tylosin	*T. intraradices* (fungus)	EC10 (hyphal lenght)	28 d	0.051	Hillis et al. 2008
erythromycin	*P. subcapitata* (green alga)	EC50 (growth)	72 h	0.020	Isidori et al. 2005
clarithromycin	*P. subcapitata* (green alga)	EC50 (growth)	72 h	0.0020	Isidori et al. 2005
tylosin	*P. subcapitata* (green alga)	EC50 (photosynteic yield)	24 h	0.0089	van der Grinten et al. 2010
erythromycin	*S. capricornutum* (green alga)	NOEC (growth)	72 h	0.010	Eguchi et al. 2004
erythromycin	*C. vulgaris* (green alga)	NOEC (growth)	72 h	13	Eguchi et al. 2004
erythromycin	*D. magna* (zooplankton)	EC50 (immobilization)	24 h	22	Isidori et al. 2005
clarithromycin	*D. magna* (zooplankton)	EC50 (immobilization)	24 h	26	Isidori et al. 2005
erythromycin	*T. platyurus* (crustacean anostraca)	LC50 (survival)	24 h	18	Isidori et al. 2005
clarithromycin	*T. platyurus* (crustacean anostraca)	LC50 (survival)	24 h	34	Isidori et al. 2005
erythromycin	*C. dubia* (crustacean cladocera)	EC50 (population growth)	7 d	0.22	Isidori et al. 2005
clarithromycin	*C. dubia* (crustacean cladocera)	EC50 (population growth)	7 d	8.2	Isidori et al. 2005
erythromycin	*B. calyciflorus* (rotifer)	EC50 (population growth)	48 h	0.94	Isidori et al. 2005
clarithromycin	*B. calyciflorus* (rotifer)	EC50 (population growth)	48 h	12	Isidori et al. 2005

Table 9: Toxicity data from different soil living and aquatic organisms exposed to quinolone drugs.

Quinolones					
Name	**Species**	**Test**	**Duration**	**Conc. (mg/l)**	**Ref.**
ciprofloxacin	sewage sludge bacteria	EC50 (growth)	48 h	0.0080	Halling-Sorensen et al. 2003
flumequine	bacterial plate Q (senitive to quinolones)	EC50 (growth)	24 h	0.20	van der Grinten et al. 2010
ciprofloxacin	*M. aeruginosa (*cyanobacteria)	EC50 (growth and reproduction)	5 d	0.017	Robinson et al. 2005
norfloxacin	*V. fischeri* (luminescent bacteria)	EC10 (bioluminescence)	24 h	0.012	Backhaus et Grimme 1999
ofloxacin	*V. fischeri* (luminescent bacteria)	EC10 (bioluminescence)	24 h	0.0039	Backhaus et Grimme 1999
ofloxacin	*M. aeruginosa (*cyanobacteria)	EC50 (growth and reproduction)	5 d	0.021	Robinson et al. 2005
enrofloxacin	*M. aeruginosa (*cyanobacteria)	EC50 (growth and reproduction)	5 d	0.049	Robinson et al. 2005
levofloxacin	*M. aeruginosa* (cyanobacteria)	EC50 (growth and reproduction)	5 d	0.0079	Robinson et al. 2005
clinafloxacin	*M. aeruginosa* (cyanobacteria)	EC50 (growth and reproduction)	5 d	0.10	Robinson et al. 2005
flumequine	*M. aeruginosa* (cyanobacteria)	EC50 (growth and reproduction)	5 d	1.96	Robinson et al. 2005
lomefloxacin	*M. aeruginosa (*cyanobacteria)	EC50 (growth and reproduction)	5 d	0.19	Robinson et al. 2005
levofloxacin	*D. carota* (fungus)	EC10 (root lenght)	14 d	0.0060	Hillis et al. 2008
levofloxacil	*T. intraradices* (fungus)	EC10 (hyphal lenght)	21 d	0.032	Hillis et al. 2008
ciprofloxacin	*P. subcapitata* (green alga)	EC50 (growth and reproduction)	3 d	19	Robinson et al. 2005
ofloxacin	P. subcapitata (green alga)	EC50 (growth)	72 h	1.4	Isidori et al. 2005
enrofloxacin	*P. subcapitata* (green alga)	EC50 (growth and reproduction)	3 d	3.1	Robinson et al. 2005
levofloxacin	*P. subcapitata* (green alga)	EC50 (growth and reproduction)	3 d	7.4	Robinson et al. 2005
clinafloxacin	*P. subcapitata* (green alga)	EC50 (growth and reproduction)	3 d	1.1	Robinson et al. 2005
flumequine	*P. subcapitata* (green alga)	EC50 (growth and reproduction)	3 d	5.0	Robinson et al. 2005
lomefloxacin	*P. subcapitata* (green alga)	EC50 (growth and reproduction)	3 d	23	Robinson et al. 2005
ciprofloxacin	*S. capricornutum* (green alga)	EC50	/	3.0	Halling-Sorensen 2000 (unpublished data)
ciprofloxacin	*L. minor* (duckweed)	EC50 (reproduction)	7 d	0.20	Robinson et al. 2005
ofloxacin	*L. minor* (duckweed)	EC50 (reproduction)	7 d	0.13	Robinson et al. 2005
enrofloxacin	*L. minor* (duckweed)	EC50 (reproduction)	7 d	0.11	Robinson et al. 2005
levofloxacin	*L. minor* (duckweed)	EC50 (reproduction)	7 d	0.051	Robinson et al. 2005
clinafloxacin	*L. minor* (duckweed)	EC50 (reproduction)	7 d	0.062	Robinson et al. 2005
flumequine	*L. minor* (duckweed)	EC50 (reproduction)	7 d	2.5	Robinson et al. 2005
lomefloxacin	*L. minor (*duckweed)	EC50 (reproduction)	7 d	0.11	Robinson et al. 2005
ofloxacin	*D. magna (*zooplankton)	EC50 (immobilization)	24 h	32	Isidori et al. 2005
ofloxacin	*T. platyurus* (crustacean anostraca)	LC50 (survival)	24 h	34	Isidori et al. 2005
ofloxacin	*C. dubia* (crustacean cladocera)	EC50 (population growth)	7 d	3.1	Isidori et al. 2005
ofloxacin	*B. calyciflorus* (rotifer)	EC50 (population growth)	48 h	0.53	Isidori et al. 2005

Table 10: Toxicity data from different soil living and aquatic organisms exposed to trimethoprim drugs.

Trimethoprim					
Name	**Species**	**Test**	**Duration**	**Conc. (mg/l)**	**Ref.**
trimethoprim	sewage sludge bacteria	EC50 (OECD test)	acc to OECD	18	Halling-Sorensen et al. 2000
trimethoprim	bacterial plate S (senitive to sulphonamides)	EC50 (growth)	24 h	0.028	Van der Grinten et al. 2010
trimethoprim	*P. phosphoreum* (bacteria)	EC50 (bioluminescence)	24 h	2.4	Jiang et al. 2010
trimethoprim	*M. aeruginosa* (cyanobacteria)	EC50 (photosynteic yield)	24 h	6.9	van der Grinten et al. 2010
trimethoprim	*P. subcapitata* (green alga)	NOEC (growh)	72 h	0.0016	Yang et al. 2008
trimethoprim	*S. capricornutum* (green alga)	NOEC (growth)	72 h	26	Eguchi et al. 2004
trimethoprim	*D. magna* (zooplankton)	EC50 (OECD test)	48 h	123	Halling-Sorensen et al. 2000
trimethoprim	*R. salina* (cryptophycean)	EC50 (growth	3 d	16	Holten Lützhoft et al. 1999

Table 11: Toxicity data from different soil living and aquatic organisms exposed to sulfonamide drugs.

Sulfonamides					
Name	**Species**	**Test**	**Duration**	**Conc. (mg/l)**	**Ref.**
sulfadiazine	sewage sludge bacteria	EC50 (growth)	10 h	15.9	Halling-Sorensen et al. 2003
sulfadiazine	sewage sludge bacteria (pour plate)	EC50 (growth)	48 h	35.4	Halling-Sorensen 2001
sulfamethoxazole	bacterial plate S (senitive to sulphonamides)	EC50 (growth)	24 h	0.052	van der Grinten et al. 2010
sulfamethoxazole	*V. fischeri* (luminescent bacteria)	EC50 (bioluminescence)	30 min	0.084	Ferrari et al. 2003
sulfamethoxazole	*M. aeruginosa* (cyanobacteria)	EC50 (photosynteic yield)	24 h	0.55	van der Grinten et al. 2010
sulfadiazine	*M. aeruginosa* (cyanobacteria)	EC50 (growth)	7 d	0.135	Holten Lützhoft et al. 1999
sulfamethoxazole	*D. carota* (fungus)	EC10 (root lenght)	28 d	0.00210	Hillis et al. 2008
sulfamethoxazole	*T. intraradices* (fungus	EC10 (hyphal lenght)	28 d	0.00210	Hillis et al. 2008
sulfamethoxazole	*P. subcapitata* (green alga)	EC50 (growth)	72 h	0.52	Isidori et al. 2005
sulfamethoxazole	*S. capricornutum* (green alga)	NOEC (growth)	72 h	0.614	Eguchi et al. 2004
sulfadiazine	*S. capricornutum* (green alga)	EC50 (growth)	72 h	2.19	Eguchi et al. 2004
sulfadimethoxine	*S. capricornutum* (green alga)	NOEC (growth)	72 h	0.529	Eguchi et al. 2004
sulfadimethoxine	*C. vulgaris* (green alga)	EC50 (growth)	72 h	11.2	Eguchi et al. 2004
sulfamethoxazole	*C. meneghinian* (alga)	NOEC (growth)	96 h	1.25	Ferrari et al. 2003
sulfamethoxazole	*S. leopolensis* (blue-green alga)	NOEC (growth)	96 h	0.059	Ferrari et al. 2003
sulfamethoxazole	*Pseudokirchneriella* (alga)	NOEC (growth)	96 h	0.09	Ferrari et al. 2003
sulfamethoxazole	*D. magna* (zooplankton)	EC50 (immobilization)	24 h	25.2	Isidori et al. 2005
sulfamethoxazole	*M. macrocopa* (zooplankton)	EC50 (immobilization)	48 h	70.4	Park and Choi 2008
sulfamethoxazole	*T. platyurus* (crustacean anostraca)	LC50 (survival)	24 h	35.36	Isidori et al. 2005
sulfamethoxazole	*C. dubia* (crustacean cladocera)	EC50 (population growth)	7 d	0.21	Isidori et al. 2005
sulfamethoxazole	*B. calyciflorus* (rotifer)	EC50 (population growth)	48 h	9.63	Isidori et al. 2005