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Review article

The urgent need for risk assessment on the antibiotic resistance spread via sewage sludge land application

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A B S T R A C T

Sewage sludge is an ever-increasing by-product of the wastewater treatment process frequently used as a soil fertiliser. To control its quality and prevent any possible hazardous impact of fertilisation, some mandatory limits of heavy metal content have been established by the European Commission (Sewage Sludge Directive). However, since the implementation of the limits, new emerging contaminants have been reported worldwide. Regardless of the wastewater treatment process, sewage sludge contains antibiotics, antibiotic-resistant bacteria and antibiotic resistance genes, which can be released into the environment through its land application. Such a practice may even boost the dissemination and further development of antibiotic resistance phenomenon – already a global problem challenging modern medicine. Due to the growing pharmaceutical pollution in the environment, the time is ripe to assess the risk for the human and environmental health of sewage sludge land application in the context of antibiotic resistance spread. In this review we present the current knowledge in the field and we emphasise the necessity for more studies.

Contents

1. Introduction

The successive implementation of the Urban Waste Water Treatment Directive 91/271/EC (European Commission, 1991) by all the European Union Members has been causing an increase in the quantity of sewage sludge - a by-product derived from the wastewater treatment process. It has been predicted that the amount of sludge generated in these countries will exceed 13 million tonnes by 2020 (Milieu, Ltd., et al., 2010). Since sewage sludge is rich in valuable nutrients and organic matter, it can be used as a fertiliser and for soil remediation purposes (Cortet et al., 2011; Weber et al., 2007). These applications appear to be an excellent solution for waste disposal. However, despite its indisputable advantages, sewage sludge contains pollutants such as heavy metals and diverse organic contaminants that may have toxic effects on all living organisms (Alvarenga et al., 2015; Passuello et al., 2010; Petrie et al., 2014). In 1986 the European Commission implemented Sewage Sludge Directive 86/278/EEC, which encouraged the application of sewage sludge in agriculture provided that the sludge is used correctly and does not impair soil quality and agricultural products. The regulated use of sludge should 'prevent harmful effects on soil, vegetation, animals and man'. To reach this goal, mandatory limit values for heavy metal

Abbreviations: ARB, antibiotic-resistant bacteria; ARGs, antibiotic resistance genes; CSA, co-selecting agents; MGEs, mobile genetic elements.

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concentrations in sludge and fertilised soils were established (Annexes IA-IC of the Sewage Sludge Directive 86/278/EEC). Moreover, since the implementation of the Directive, untreated sludge must not be applied, unless it is injected or worked into the soil (European Commission, 1986). Treated sewage sludge, often referred to as biosolids, is subjected to additional processes (such as aerobic or anaerobic digestion) prior to land application in order to minimise the content of pathogenic bacteria (Youngquist et al., 2014).

Because of new emerging contaminants, there is a growing debate about the revision of the Sewage Sludge Directive. A study launched by the European Commission revealed emerging risks connected to sludge use on land. The FATE SEES (FATE — fate and impacts of pollutants in terrestrial and aquatic ecosystems, SEES — sewage sludges and effluents for emerging substances) monitoring project is dedicated to assessing the presence and concentration levels of pollutants in sewage sludge. Aside from the well-established contaminants such as heavy metals, polychlorinated biphenyls, polychlorinated dibenzo-*p*-dioxins and polycyclic aromatic hydrocarbons, the study is focused on the less-investigated emerging pollutants such as brominated flame retardants, the ingredients of personal care products, and last but not least, pharmaceuticals (Gorga et al., 2013; Gurke et al., 2015; Roig et al., 2012).

The occurrence of pharmaceuticals in the environment and their potential ecotoxicological effects have drawn special attention in recent years (Malmborg and Magner, 2015; Martín et al., 2015). Drugs are not completely metabolised in human and animal bodies and they are excreted either as parent compounds or as metabolites via urine and faeces. They may enter the environment directly along with the faeces, (e.g., through land application of manure) or via wastewater treatment plants effluents and the application of sewage sludge (Gao et al., 2012; Loganathan et al., 2009; McEneff et al., 2014). Due to the global spread of antibiotic resistance among bacteria, the release of antibiotics, antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) is a matter of special concern (Roca et al., 2015). Furthermore, the co-occurrence of antibiotic and metal resistance in bacteria has been observed (Máthé et al., 2012). This effect is caused by the cross- and co-resistance phenomena. Cross-resistance occurs when the same mechanism reduces the susceptibility to metals and antibiotics simultaneously and co-resistance occurs when separate resistance genes are situated on the same genetic element (Baker-Austin et al., 2006; Knapp et al., 2011). This fact may be of great importance in the case of the agricultural use of sewage sludge, since as was already mentioned, it contains significant amounts of heavy metals (Alvarenga et al., 2015; Heck et al., 2015; Milinovic et al., 2014; Seiler and Berendonk, 2012). Therefore, the presence of heavy metals in both sludge and sludge-amended soil may select for antibiotic-resistant bacteria in fertilised soil (Baker-Austin et al., 2006; Gullberg et al., 2014; Seiler and Berendonk, 2012).

The aim of this review is to summarise recent advances in the field of land application of sewage sludge in the context of antibiotic resistance phenomenon.

2. Antibiotic resistance in sewage sludge

It is well established that sewage sludge contains significant amounts of diverse antibiotics that represent nearly all major classes (excluding labile β-lactams). The concentrations assessed in sewage sludge vary between ng to mg per kg of dried weight (Jelić et al., 2012; Le-Minh et al., 2010; Li et al., 2013; McClellan and Halden, 2010). It has been documented that pharmaceuticals are adsorbed on sewage sludge particles. The rate of this process depends on drug chemical structure, mobility, hydrophobicity, biodegradation and the nature of the sludge itself. Moreover, it has been reported that pharmaceuticals adsorbed to sludge exhibit a higher degree of stability than those found in wastewater (Cheng et al., 2014; Li et al., 2013). Recent findings suggest that even ppb (part per billion) concentrations of antimicrobials maintain the ARGs in bacterial populations and may favour plasmid transfer (Gullberg et al., 2014; Kim et al., 2014). Due to antibiotic content, it is reasonable to assume that some microorganisms residing in sewage sludge may be intrinsically tolerant to these compounds (e.g. due to cell envelope impermeability) and/or exhibit antibiotic resistance conferred by clinically relevant mechanisms. Indeed, regardless of applied method treated sewage sludge is rich in ARB. However, the most advanced technologies such as anaerobic digestion and lime stabilisation significantly reduce ARB number when compared to simple dewatering and gravity thickening (Munir et al., 2011). Intriguingly, even if ARB lose viability during the treatment process, the frequency of ARGs may simultaneously increase. Studies conducted by Su et al. (2015) revealed 156 unique ARGs and mobile genetic elements encoding resistance to virtually all of the known antibiotic groups in composted sewage sludge, thus suggesting this by-product is a significant reservoir of antibiotic resistance determinants (Calero-Cáceres et al., 2014; Su et al., 2015).

3. The persistence of antibiotic-resistant bacteria in fertilised soils assessed by culture-based methods

Antibiotics are abundant in wastewater and are not completely eliminated during the treatment process. They persist in activated sludge basins at sublethal concentrations, thus leading to the selection of resistant bacteria. The presence of antibiotics together with a high microbial cell density and diversity favour the horizontal gene transfer (HGT) of resistance determinants among the bacteria residing in activated sludge (Michael et al., 2013; Zhang et al., 2011). Antibiotic-resistant bacteria and antibiotic resistance genes may subsequently be transferred to the environment through the land application of sewage sludge (Hölzel et al., 2010; Rahube et al., 2014; Riber et al., 2014).

Culture-based approaches were used to determine the persistence of antibiotic-resistant bacteria in sewage sludge-fertilised soils (Table 1). Even though the culture-based methods allow only a small fraction of the bacteria present in soil to be grown (ca. 1%), they still provide valuable information about the resistance phenomenon. Culture-based methods give the possibility to establish the link between antibiotic resistance genes and their bacterial hosts. Well-known pathogenic and indicator bacteria are easily grown on selective media using standard procedures. That is why assessing potential human health risks is feasible using culture-based approaches. Furthermore, phenotyping provides information about complete and active genes, which is not always the case when using molecular methods such as qPCR. On the other hand, culture-dependent methods are laborious and time consuming, nevertheless their major limitations stem from the narrow fraction of bacteria that can be cultivated (Hill et al., 2000; Torsvik et al., 1998).

In order to get information about the fate of culturable ARB in fertilised soils, two approaches may be applied. One considers the persistence of ARB added to soil together with sewage sludge, while the second screens for antibiotic resistance among autochthonous microorganisms. It is expected that although the number of bacteria carried with fertiliser would decrease with time, some changes in ARGs pool among soil-dwelling bacteria might be observed.

A three-year study conducted by Rahube et al. (2014) added significant information to the current knowledge in this field. The authors investigated the impact of fertilising with raw sewage sludge and dewatered municipal biosolids (anaerobically digested sewage sludge) on the abundance of ARB, pathogens and ARGs in soils and on vegetables at harvest. In this comprehensive field study, the presence of pathogenic bacteria and antibiotic-resistant coliforms was verified using culture-dependent methods. As expected, the number of viable bacteria evaluated in the sewage sludge was much higher when compared with the biosolids. None of the pathogens detected and quantified in the sewage sludge were found at quantifiable levels in the biosolids. The percentage of antibiotic-resistant coliform bacteria was also higher in