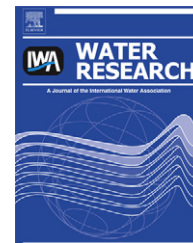




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Review

The potential for a suite of isotope and chemical markers to differentiate sources of nitrate contamination: A review

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ABSTRACT

Nitrate is naturally found within the environment as part of the nitrogen cycle. However, anthropogenic inputs have greatly increased nitrate loads within ground and surface waters. This has had a severe impact on aquatic ecosystems and has given rise to health considerations in humans and livestock. Therefore, the identification of nitrate sources is important in preserving water quality and achieving sustainability of our water resources. Nitrate sources can be determined based on the nitrate nitrogen (N) and oxygen (O) isotopic compositions ($\delta^{15}\text{N}$, $\delta^{18}\text{O}$). However, sewage and manure have overlapping $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values making their differentiation on this basis problematic. The specific differentiation between sources of faecal contamination is of particular importance, because the risk to humans is usually considered higher from human faecal contamination (sewage) than from animal faecal contamination. This review summarises the current state of knowledge in using isotope tracers to differentiate various nitrate sources and identifies potential chemical tracers for differentiating sewage and manure. In particular, an in depth review of the current state of knowledge regarding the necessary considerations in using chemical markers, such as pharmaceuticals and food additives, to differentiate sewage and manure sources of nitrate contamination will be given, through an understanding of their use, occurrence and fate, in order to identify the most suitable potential chemical markers.

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Abbreviations: FIB, faecal indicator bacteria; WWTP, wastewater treatment plant; BOD, biological oxygen demand.

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1. Introduction

The nitrate ion (NO_3^-) occurs naturally as part of the nitrogen cycle. However, its ever-increasing concentrations have made it a ubiquitous contaminant of natural water resources. Nitrate arises from several point and non-point (diffuse) sources including synthetic and natural fertilisation, bacterial production, atmospheric deposition and leaking septic systems (Bordeleau et al., 2008). In addition, biogeochemical processes are known to modify nitrate concentrations such that different forms of nitrogen (NO_2 , NH_4 , NH_3) can potentially be transformed into nitrate (WHO, 2004).

Nitrate is considered to be a contaminant of concern because its presence within the environment has been linked to various environmental and health considerations. Such concerns have led to several pieces of legislation aimed at limiting nitrate concentrations. Within the European Union, these may be found in several pieces of legislation (Bouraoui et al., 2009), such as the Nitrates Directive (91/676/EEC), the Urban Wastewater Directive (91/271/EEC), the Water Framework Directive (2000/60/EC) and the Groundwater Directive (2006/118/EC). Other measures related to nitrate contamination include the Common Agricultural Policy reform, whereby subsidies have been decoupled from production levels and linked to the application of statutory minimum requirements and cross compliance leading to a decrease in fertiliser use (Bouraoui et al., 2009). However, at this point in time, there is no limit set for nitrates in rivers or lakes (EPA, 2009).

Determining the sources of nitrate contamination in water bodies and understanding the processes affecting local nitrate concentrations are necessary for a number of reasons. These include:

1. Improved management of water bodies for preserving water quality;
2. Actions for the remediation of contaminated sites can be targeted to the actual source making them more efficient, thus reducing public health and environmental considerations related to elevated nitrate concentrations;
3. More effective application of the 'polluter pays principle' in the context of nitrate contamination, since the inputs can be identified (Kendall, 1998; Kraft and Stites, 2003; Curt et al., 2004; Xue et al., 2009).

Unfortunately, the sources of nitrate within different regions may vary considerably even on a small scale (EEA, 2005). The relationship between nitrate concentrations in groundwater and surface water, and the quantity of nitrate introduced from a specific source is complicated by a number of factors. These include the occurrence of multiple inputs, the presence of overlapping point and non-point sources, the coexistence of several biogeochemical processes that alter nitrate concentrations, the presence of various factors affecting nitrate concentrations (such as human activity, geography and climate) and the occurrence of considerable temporal variations dependent upon precipitation levels leading to inter-annual variations (Kendall, 1998; Curt et al., 2004; EEA, 2005; Chen et al., 2010).

The present review provides an overview of the current state of knowledge in using isotope tracers to differentiate various sources. It then focuses on identifying potential chemical tracers for differentiating sewage and manure. In particular, an in depth review of the current state of knowledge concerning the necessary considerations in using pharmaceuticals and related compounds, such as food additives as chemical markers, to differentiate sewage and manure sources of nitrate contamination will be given. The specific differentiation between sources of faecal contamination is of particular importance as the risk to humans is usually considered to be higher from human faecal contamination (sewage) than from animal faecal contamination since viruses, which represent an important basis of illness resulting from faecal exposure, are highly host specific (Field and Samadpour, 2007).

2. Methods of differentiating nitrate sources

In an effort to distinguish between sources of nitrate contamination, various approaches have been adopted. These have been applied with different degrees of success for source determination. However, to the authors' knowledge, no one technique has been determined to be suitable to differentiate all sources of nitrate contamination. Furthermore, it is likely that a suite of techniques and indicators must be utilised in conjunction with each other in order to achieve successful differentiation.

Two of the main areas of research in nitrate source determination are the use of isotope tracers and chemical markers. The use of isotopic tracers generally represents the technique of choice in differentiating broad classes of nitrate contamination. However, it is not suitable in differentiating closely related sources of nitrate contamination, such as sewage and manure.

A wider range of approaches have then been utilised in achieving faecal source tracking to track sewage and manure inputs, including antibiotic resistance (Carroll et al., 2005), biochemical fingerprinting (Blanch et al., 2006), DNA fingerprinting (Edge et al., 2007), bacteriophage occurrence (Vijayavel et al., 2010) and the use of genetic markers (Scott et al., 2005). An in depth review of these methods and their suitability is available elsewhere (Field and Samadpour, 2007). However, many of the methods outlined have not been fully tested to the stage of application in field studies and a number of concerns have been raised in relation to their use. For example, the contamination of a water body by sewage or manure is generally determined using the detection of faecal indicator bacteria (FIB). However, whilst it is useful for the detection of sewage and manure inputs as a class, it is currently not possible to distinguish between human or animal sources on this basis, as FIB such as *Escherichia coli* and enterococci do not discriminate between human and animal faecal matter sources (Glassmeyer et al., 2005; Young et al., 2008; Gourmelon et al., 2010). Therefore, it does not provide additional characterisation to that provided by the dual-isotope method. For this reason, other tracers must be used to achieve this differentiation.

One way of doing this is to use isotopic techniques in conjunction with chemical markers as a suitable method for the determination of sources of nitrate contamination and for further differentiation of sewage and manure inputs. Various chemical markers have been suggested as being suitable indicators of faecal contamination. Their use in combination with nitrate isotope data could hence be successful in discriminating between the nitrate inputs into a water body.

Chemical markers of faecal contamination can fall into several classes, namely those that are produced by humans e.g. the faecal sterol coprostanol, those that pass through humans e.g. pharmaceuticals and food additives, and those that are associated with the sewage contaminated waste system e.g. detergents (Glassmeyer et al., 2005; Kasprzyk-Hordern et al., 2009).

Ideal chemical markers of faecal contamination (sewage or manure) are ubiquitous in the source and derive only from the particular source, persistent and present at detectable concentrations in contaminated environmental samples but not in clean water, and have a high water solubility, low K_w and low volatility allowing for their use as tracers for water-soluble components originating in sewage and manure (Kasprzyk-Hordern et al., 2009). Therefore, chemical markers such as pharmaceuticals and food additives have been considered more desirable, than others such as fragrances and detergents, since they are ingested and would be excreted from the consumer indicating a direct relationship (Glassmeyer et al., 2005).

Pharmaceuticals and related compounds are known to have numerous properties favouring their use as chemical

tracers. Their physiochemical properties suggest that they are relatively water-soluble and non-volatile (Lacey et al., 2011), and their natural background levels are low (Benotti and Brownawell, 2007). In addition, ever-decreasing detection limits are currently allowing for their detection even when the source water is substantially diluted (Benotti and Brownawell, 2007). Furthermore, they are commonly persistent in order to avoid the substance becoming inactive before having a curing effect (Christensen, 1998; Halling-Sørensen et al., 1998; Jjemba, 2002, 2006; Anderson et al., 2004; Enick and Moore, 2007).

Presently, chemicals found in wastewaters, are increasingly being suggested as chemical markers for the verification of water contamination with sewage (Glassmeyer et al., 2005; Kasprzyk-Hordern et al., 2009). However, by the careful selection of chemicals showing human or animal source specificity, it is expected that it is possible to distinguish between sewage and manure nitrate contamination.

3. Use of isotope tracers

Natural and anthropogenic isotopes have been used to explore a number of hydro geochemical issues as a function of their wide distribution (Kendall and Caldwell, 1998). For a particular isotope to be useful as a tracer, the relative mass difference of common to rare isotopes of the element should be large. In addition, the rare isotope must be present at substantial levels yet at significantly lower levels to the more abundant isotope (Toran, 1982). Theoretical aspects of the isotopic tracers are described elsewhere (Kendall and McDonnell, 1998; Xue et al., 2009), and therefore this review paper will focus on giving a short background to the use of isotope tracers, and in particular the factors that allow the use of isotopes to be used as tracers, and also in putting more recent literature in perspective.

A typical use of isotope tracers is in the identification of contaminant sources. This is possible for a variety of reasons, including:

1. Waters originating at different times and locations often have distinctive isotopic compositions;
2. Environmental isotopes are not normally considered to react significantly with catchment materials;
3. Changes in solute isotopic ratios generally occur in predictable and recognisable directions, hence allowing them to be reconstructed from the isotopic compositions (Kendall and Caldwell, 1998; Kendall, 1998).

Isotopic fractionation, resulting in distinctive isotopic compositions, is the underlying cause for the application of isotope tracers in source determination. Isotopic fractionation is the division of heavy and light isotopes disproportionately between reaction substrates and products. It occurs because atomic masses and bond strengths are isotope dependent (Cravotta, 1997). Hence, isotopes of the same element would have slightly different chemical and physical properties, which can result in mass-dependent isotope fractionation (Kendall and Caldwell, 1998). These effects are of greater

consequence at lower temperatures and disappear with increasing temperatures (Kendall and Caldwell, 1998).

Isotopic fractionation can occur through reversible equilibrium reactions or irreversible unidirectional kinetic reactions (Kendall, 1998). Equilibrium fractionations are generally associated with physiochemical reactions (Böhlke, 2003). During equilibrium reactions, heavier isotopes generally accumulate preferentially in the species or compound having the higher oxidation state (Kendall and Caldwell, 1998). Kinetic (unidirectional) isotope fractionation results in depleted products (Mariotti et al., 1981). This has been attributed to increased stability of molecules containing the heavy isotope because of higher dissociation energies than molecules with lighter isotopes (Mariotti et al., 1981; Kendall and Caldwell, 1998). Kinetic fractionations are associated with both physiochemical and biochemical reactions (Böhlke, 2003). The extent of kinetic isotope fractionation depends on the reaction pathway, the reaction rate and the relative bond energies of the bonds being severed or formed by the reaction (Kendall and Caldwell, 1998).

Therefore, the isotopic composition of a particular water body not only reflects the composition of the original source or of mixed sources having different compositions but can be influenced by isotopic fractionation during the transport and chemical transformation of the compounds (Cravotta, 1997; Kellman and Hillaire-Marcel, 1998; Nestler et al., 2011). Nitrate is a typical contaminant for which isotope tracer techniques have been applied (Xue et al., 2009). This is possible since most nitrogen (N) sources are interrelated in the biochemical N cycle and measurable differences in the isotopic composition of N-source materials persist as N-containing compounds are transported from the source (Cravotta, 1997). Furthermore, both N and O within nitrate have naturally occurring stable isotopes, which may be exploited in isotope tracer studies.

3.1. $\delta^{15}\text{N}$ of nitrate sources

The wide range of oxidation numbers exhibited by nitrogen compounds, ranging from +5 (NO_3^-) to -3 (NH_4^+), results in

a wide natural range of isotopic compositions (Kendall, 1998). This assists in differentiation of sources. $\delta^{15}\text{N}$ compositions of most terrestrial materials fall between -10‰ and $+25\text{‰}$ (Fig. 1) (Kendall, 1998).

The use of nitrate isotopes to trace nitrate sources has been determined to be applicable even where nitrogen inputs from the different sources can be estimated and the outputs into the surface water body can be measured. This is because the physical, chemical and biological processes that control nitrogen-cycling act unequally upon nitrogen from different sources and, therefore, the different sources may contribute nitrogen disproportionately to their inputs within the catchment (Fig. 2) (Battaglin et al., 1997).

The variations in $\delta^{15}\text{N}$ values, hence allows for the discrimination of a number of nitrate sources. Yet, numerous other sources cannot be distinguished on this basis. For example, sewage and manure nitrate have overlapping values, which makes their differentiation using $\delta^{15}\text{N}$ values problematic. It has been suggested that nitrate in seepage from septic systems is at the lower end of the range ($+8\text{‰}$ to $+11\text{‰}$) whilst nitrate in leachate from manure spreading occur at a higher range ($+10\text{‰}$ to $+25\text{‰}$) and shows more variability (Böhlke, 2003). However, the occurrence of relatively high and variable $\delta^{15}\text{N}$ values in waters beneath septic systems and manure spreading areas (Böhlke, 2002) and the considerable fractionation that occurs makes human and other animal waste isotopically indistinguishable in most circumstances (Kendall, 1998; Xue et al., 2009).

3.2. The dual isotope approach

The difficulty in conclusively differentiating the numerous nitrate sources, including sewage and manure, based solely on the $\delta^{15}\text{N}$ isotopic compositions, has led to the application of a dual isotope approach. This involves the determination of both nitrogen and oxygen isotopic compositions. The dual isotope approach has three main potential benefits (Kendall, 1998):

1. Oxygen isotopic separation of some sources is greater than for nitrogen isotopes with the spread of $\delta^{18}\text{O}$ being at

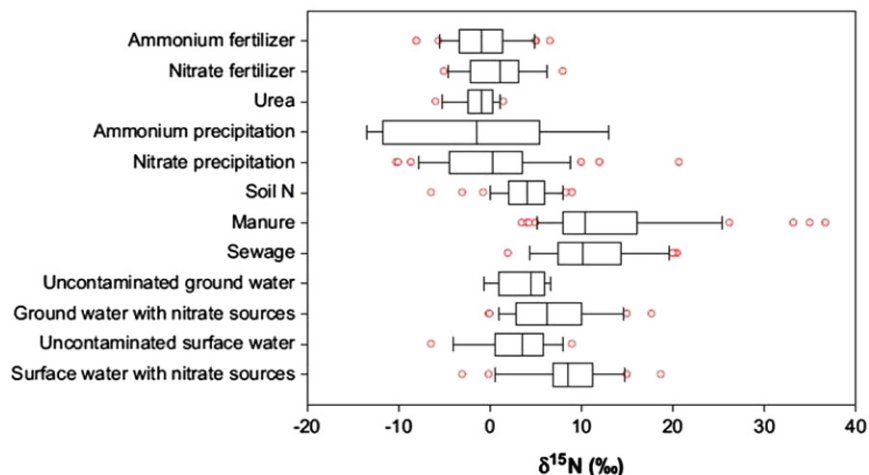


Fig. 1 – Box plots showing 25th, 50th and 75th percentiles for $\delta^{15}\text{N}$ values of nitrate from various sources and sinks. The whiskers show the 10th and 90th percentiles, and the circles represent outliers (Xue et al., 2009).

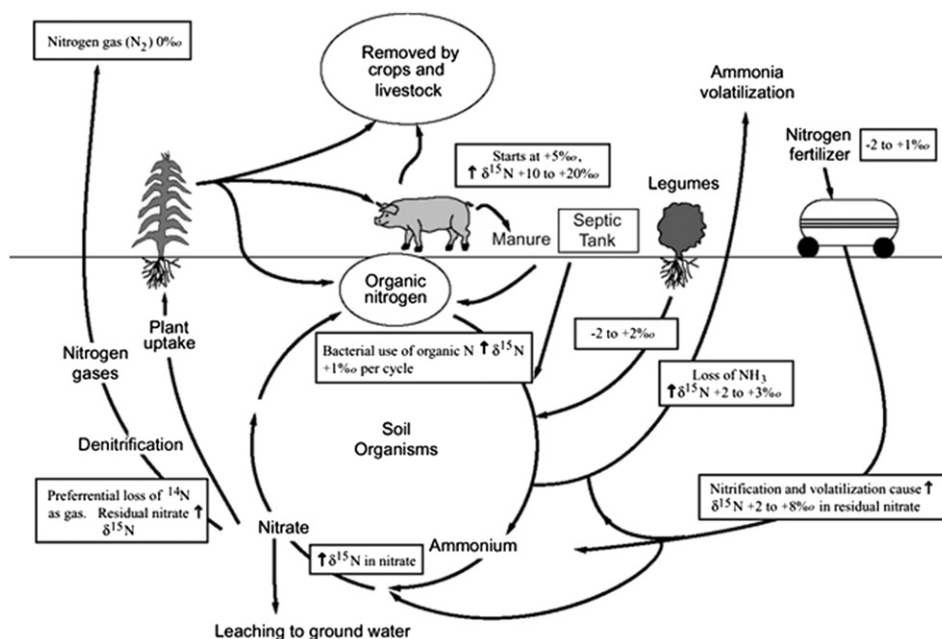


Fig. 2 – Range of $\delta^{15}\text{N}$ values for various sources of nitrogen and the effects of different processes (Townsend, 2008).

around 80‰, whilst that for $\delta^{15}\text{N}$ being at 35‰, thus, allowing better source resolution by having two tracers (Fig. 3);

- Some nitrate sources that are presently indistinguishable with $\delta^{15}\text{N}$ alone (e.g. fertiliser vs soil nitrate, or atmospheric vs soil nitrate) may be identified once $\delta^{18}\text{O}$ values are incorporated;
- Oxygen isotopic compositions of nitrate vary systematically with nitrogen isotopic compositions during denitrification. Thus, in systems where the dominant sources of nitrate are isotopically distinctive, source contributions can (in theory) be determined despite significant denitrification.

In a similar manner to $\delta^{15}\text{N}$, a number of factors have been found to alter the $\delta^{18}\text{O}$ isotopic values from the source to the sink, which is not simply a result of the averaging out of the different source values.

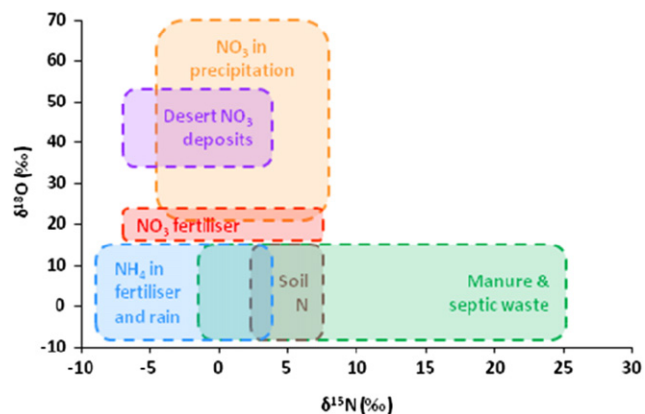


Fig. 3 – A general depiction of the normal range of $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values for the dominant sources of nitrate. Adapted from (Kendall, 1998).

To date, the dual isotope approach has been applied successfully to numerous scenarios such as hydrologic studies into the transformation pathways of nitrate and, in particular, denitrification (Böttcher et al., 1990; Aravena et al., 1993; Aravena and Robertson, 1998; Cey et al., 1999; Battaglin et al., 2001; Chang et al., 2002; Fukada et al., 2003; Lehmann et al., 2004; Baulch et al., 2011; Itoh et al., 2011). Most studies have focused on the identification and quantification of diffuse nitrate inputs into a water body (Wassenaar, 1995; Chang et al., 2002; Mayer et al., 2002; Deutsch et al., 2006; Bordeleau et al., 2008; Lee et al., 2008; Kaown et al., 2009; Wankel et al., 2009; Palau and Soler, 2010; Kaushal et al., 2011). The dual isotope approach has also been applied to studies on the effects of forest decline on the uptake and leaching of deposited nitrate (Durka et al., 1994), and in studies on seasonal variations of dissolved nitrate (Lee et al., 2008; Buda and DeWalle, 2009; Savard et al., 2010).

Unfortunately, in the case of sewage and manure, there is no contribution of $\delta^{18}\text{O}-\text{NO}_3^-$ data for nitrate source identification (Xue et al., 2009; Nestler et al., 2011). Such a difficulty is likely due to the similarity between the isotopic composition of nitrate arising from animal (humans included) waste undergoing similar fractionation pathways.

In conclusion, whilst the dual isotope approach is indeed useful for the differentiation of numerous nitrate sources, for example nitrate and ammonia fertiliser, which is not possible using $\delta^{15}\text{N}$ values in isolation. This method is not suitable for the discrimination between sewage and manure nitrate sources.

3.3. Other geochemical tracers

The differentiation between sewage and manure nitrate has been attempted in numerous ways, including linking $\delta^{15}\text{N}$ and

$\delta^{18}\text{O}$ values with land used types or physico-chemical properties of water, such as pH, conductivity, ammoniacal and nitrate nitrogen concentrations, chloride and the levels of dissolved organic and inorganic carbon and nitrogen (Karr et al., 2001; Mayer et al., 2001; Spruill et al., 2002; Mitchell et al., 2003; Kaushal et al., 2006; Bu et al., 2011; Ma et al., 2011). Boron isotopes have also been used for the identification of nitrate sources in groundwater (Bassett et al., 1995; Komor and Anderson, 1993; Vengosh et al., 1999; Widory et al., 2004, 2005). However, as with nitrogen and oxygen isotopes, the range of these isotopic compositions for sewage and manure overlap, making them difficult to differentiate on this basis (Nestler et al., 2011).

Overall, no fully conclusive method of differentiation between sewage and manure nitrate within surface waters has yet been published.

4. Use of chemical markers

In this section, a review of the currently available data on the use of chemical markers and, in particular, pharmaceuticals, and related chemicals such as food additives, to discriminate between sewage and manure contamination and the specific considerations in developing a suite of chemical markers is discussed.

The choice of the particular suite is of critical importance in the use of pharmaceuticals as chemical markers. In particular, an understanding of their use within the community being studied (Section 4.1), their fates on being transported from the source to surface waters (Section 4.2) and their occurrence within surface waters (Section 4.3).

4.1. Use of pharmaceuticals in human and veterinary treatment

The development of pharmaceuticals has substantially improved human and animal health through the introduction of compounds acting as analgesics and anti-inflammatory drugs, contraceptives, antibiotics, beta-blockers, lipid regulators, neuroactive compounds, condition-specific formulations etc. (Cahill et al., 2004). The consumption of pharmaceuticals has reached substantial levels and are now being considered emerging environmental micro contaminants because of their potential adverse effects on ecosystems and human health (Arikan et al., 2008).

In the EU, over 3000 different substances are used in human medicine (Petrovic et al., 2008). A similar number of

pharmaceuticals are used in veterinary medicine (Fent et al., 2006). Current livestock production involves ubiquitous use of different chemicals including antibiotics, parasiticides, antifungals, hormones and metals (feed additives) to treat disease, protect the health of animals and improve animal productivity (Heberer, 2002; Anderson et al., 2004; Boxall et al., 2006).

Although pharmaceuticals are generally not high-production-volume chemicals and the expected environmental concentrations may consequently be assumed to be low (ng L^{-1} range, Table S1), because of their pervasive use, pharmaceuticals and their metabolites have been inadvertently introduced into surface and ground waters (Cahill et al., 2004). Their presence in the environment was first noted more than 30 years ago (Hignite and Azarnoff, 1977). However, the increase in attention to their discharge, presence and potential adverse effects on the environment is largely a recent effort of the past decade. Most of the studies carried out to date have focused on human pharmaceuticals. However, the recent intensification of animal production, in particular the proliferation of large-scale animal feeding operations during the last decade (Tvrčko, 2008), has resulted in increased water quality concerns related to the production and disposal of animal waste generated by these operations (Arikan et al., 2008).

In determining the suite of pharmaceuticals appropriate for differentiating sewage and manure inputs, it is particularly important, to note that a number of potential chemical markers are consumed by both humans and livestock e.g. antimicrobial agents such as some tetracyclines and sulfonamides (Kasprzyk-Hordern et al., 2009). The list of approved pharmaceuticals may vary between countries and therefore pharmaceutical use can differ depending upon the community being studied. This makes it important to understand their use and application within a particular country prior to adopting a suite of pharmaceuticals and related compounds as chemical markers for distinguishing between sewage and manure nitrate inputs into surface waters. Some examples of pharmaceuticals which are used for the treatment of humans, animals or both are given in Table 1 indicating suitable indicators of human and animal sources.

4.2. Fate of pharmaceuticals within the aquatic environment

Pharmaceuticals and related compounds are known to enter the aquatic environment through a variety of routes (Fig. 4), the main difference being whether the pharmaceutical was intended for human or veterinary use. This difference could

Table 1 – Examples of pharmaceuticals which are used solely for human or veterinary treatment and others which are used for both human and veterinary treatment (IMB, 2010a; IMB, 2010b; VMD, 2010; NOAH, 2011).

Use	Examples
Human treatment	Acetaminophen, caffeine, carbamazepine, codeine, diltiazem, diphenhydramine, ibuprofen, propranolol, meclufenamic acid, gabapentin
Veterinary treatment	Enrofloxacin, tylosin, sulfadimethoxine, lincomycin, doramectin, tilmosin, ivermectin, diazinon, cypermethrin, cloxacillin, sulfadiazine
Human and veterinary treatment	Cimetidine, ketoprofen, sulfamethoxazole, thymol, amoxicillin, ampicillin, erythromycin, neomycin, trimethoprim, oxytetracycline, tetracycline

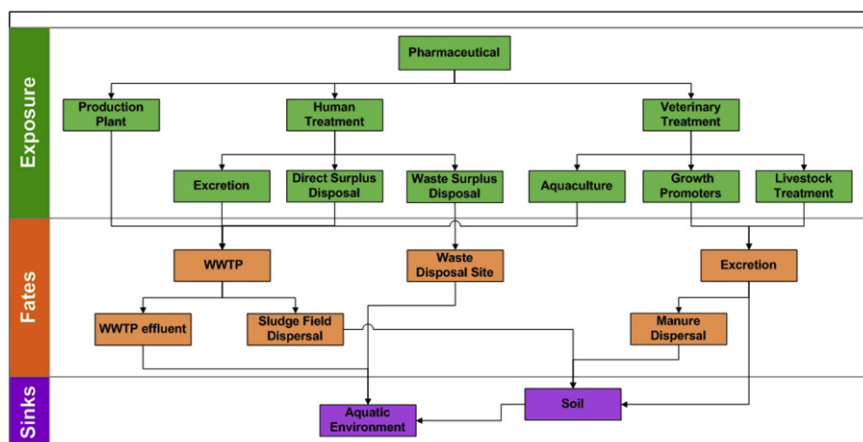


Fig. 4 – Scheme indicating the sources and pathways resulting in the presence of pharmaceuticals in the aquatic environment. Adapted from (Jørgensen and Halling-Sørensen, 2000; Heberer, 2002; Ziylan and Ince, 2011).

potentially be exploited in the use of such chemical markers to differentiate sewage and manure. Understanding the fate of pharmaceuticals from source to sink is important in determining their applicability as part of a suite of chemical markers for differentiating sewage and manure.

4.2.1. Exposure

Excretion after normal pharmaceutical use is a major environmental pathway for many pharmaceuticals. How the pharmaceutical is emitted during treatment depends upon the manner of administration. For example, treatment may be received topically, orally or as an injection (Boxall et al., 2003; Mehta et al., 2010). After administration, excretion may be as an unchanged parent compound, in the form of metabolites, or as conjugates of glucuronic and sulfuric acid (Calamari et al., 2003; Carballa et al., 2004; Petrovic et al., 2008). However, excretion levels of the unchanged parent compound have been estimated to be up to 90% for some compounds (Sarmah et al., 2006).

Metabolisation pathways of pharmaceuticals vary. Most pharmaceuticals are metabolised to phase I or phase II metabolites (Fig. 5) before being excreted from the body in humans and other mammals (Halling-Sørensen et al., 1998). Both phase I and phase II reactions, act to change the physico-chemical behaviour of the substance and, particularly, metabolisation renders the metabolites more water soluble than the parent compounds (Halling-Sørensen et al., 1998). Because of this, pharmaceutical metabolites are expected to be even more persistent in the environment, due

to their increased polarity. In addition, some metabolites may be converted back into the original drug during sewage treatment (Petrovic et al., 2008). For example, the metabolites of chloramphenicol, sulfadiazine, oestrogen and sulfamethazine can be converted back to the parent compound.

Hence, the presence of metabolites may be exploited as chemical markers in instances where the degree of metabolism is high resulting in extremely low concentrations of the parent compound. For example, while unipolar lipid regulators clofibrate, etofibrate and fenofibrate are not detectable in WWTP effluents, their polar metabolites clofibric acid and fenofibric acid have been detected in effluents (Ternes, 1998; Jjemba, 2002).

Disposal of unused and expired pharmaceuticals is another route of entry. Pharmaceuticals would enter the solid waste stream or sewage stream in an unmodified form having avoided metabolism in the body. This could make their contribution to environmental contamination disproportionate. Nevertheless, disposal of unused medicines with sewage appears to be of minor importance, while patient excretion following therapy is widely considered the primary pathway to the environment (Heberer, 2002; Anderson et al., 2004). However, such disposal of unused and expired pharmaceuticals is relevant to both human and veterinary compounds, as is the release from industrial production of pharmaceuticals (Deegan et al., 2011) and, thus, these inputs must be considered in the application of chemical markers to differentiate sewage and manure.

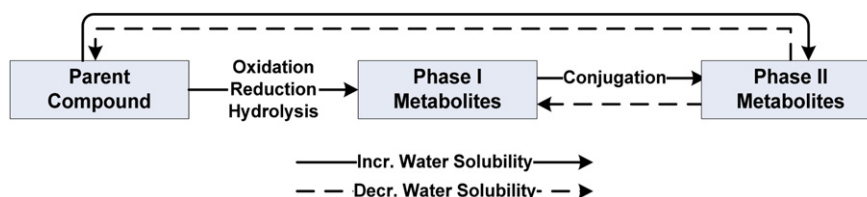


Fig. 5 – An overview of metabolic transformations of pharmaceuticals and the resulting changes in solubility and polarity. Adapted from (Halling-Sørensen et al., 1998; Daughton and Ternes, 1999).

4.2.2. Fates and transport

The fate and transport of human and veterinary wastes largely depend upon the original source. On excretion, human waste streams are generally diverted towards a WWTP where the resultant sewage stream is treated before being released into discharging water as effluent (Ternes, 1998). Around 80% of EU wastewater passes through a WWTP before being discharged into surface waters, but the number of inhabitants per WWTP and the operating technical standards at the WWTP are highly variable (Ternes, 1998; O'Brien and Dietrich, 2004). Therefore, the efficacy of WWTPs in removing xenobiotics, such as pharmaceuticals, may vary widely (O'Brien and Dietrich, 2004; Lacey et al., 2011). The type of treatment involved is a major determinant, with different pharmaceuticals being affected in diverse manners after undergoing different treatments (Ternes, 1998; Golet et al., 2001).

Studies have suggested that as much as 80% of the total load of pharmaceuticals entering WWTPs is discharged in effluents unchanged (Oulton et al., 2010). For example, Andreozzi et al. (2003), reported that many pharmaceuticals (all antibiotics, gemfibrozil, fenofibrate, ibuprofen, naproxen, diclofenac, carbamazepine and most β -blockers studied) were detected in almost every sample of WWTP effluent analysed from four European countries (Italy, France, Greece and Sweden). This is most likely a result of their high prescription extent and wide usage in Europe. Other routes of human pharmaceutical entry into the aquatic environment include the use of sewage sludge as fertiliser or treated wastewater for irrigation in agriculture.

Veterinary pharmaceuticals can enter the environment in a more direct manner compared to human drugs. This is particularly true for pharmaceuticals used in aquaculture, which are released directly into the surface water (Jjemba, 2006). Furthermore, unlike sewage, which is usually treated, animal excrement from intensively reared livestock is usually piled, composted or stored as a slurry in manure tanks, lagoons or pits without any deliberate treatment (Jjemba, 2002; Boxall et al., 2006). The manure from medicated animals is then typically managed in the same way as manure from unmedicated animals (Arikan et al., 2007). Animal manure tends to be highly concentrated and has a higher biological oxygen demand (BOD) compared to treated sewage sludge (Jjemba, 2002). Under such conditions of high BOD, the existing therapeutic agents in manure are even less likely to be degraded (Jjemba, 2002). The field application of manure from treated animals may cause their release to the soils during the slurry or manure application process and subsequently transported to surface or groundwater via surface runoff or leaching (Boxall et al., 2006).

Pharmaceuticals used in animals raised on pastures are excreted directly to the grassland if applied orally or by injection. If the pharmaceutical has been applied topically, it can be washed off (Boxall et al., 2006). Pharmaceuticals, on entering the terrestrial environment, can reach surface water and groundwater via direct runoff of on-ground faecal material or through leaching.

Of note is that livestock waste treatment plants (mainly based on anaerobic digestion) have been developed and would present another source of veterinary pharmaceutical

contamination (Kim et al., 2008; Martinez et al., 2009). Such treatment plants function in a similar way to sewage WWTPs and, hence, the same considerations regarding degradation apply.

4.2.3. Persistence within the environment

Regardless of the source of pharmaceuticals, the compound's persistence within the environment is a determining factor for the chemical marker's ultimate fate. Both abiotic and biotic mechanisms act to determine the fate of organic compounds in the aquatic environment. The ultimate fate of pharmaceuticals may be divided into three different routes:

1. Substance is ultimately mineralised to carbon dioxide and water;
2. Substance is lipophilic and not readily degradable so that part of the substance will be retained;
3. Substance is metabolised to a more hydrophilic form than the parent lipophilic substance but persists to pass to the receiving waters (Halling-Sørensen et al., 1998).

Pharmaceuticals that are readily mineralised to carbon dioxide and water are largely unsuitable for use as chemical tracers as these would not be detected within the environment. In order for pharmaceuticals to be suitable as chemical tracers, their degradation must be limited or, alternatively, form relatively stable transformation products, which could be detected within the environmental phase of interest.

In general, both abiotic and biotic mechanisms act to determine the fate of organic compounds in the aquatic environment. Biodegradation, sorption and photodegradation are recognised to be the major pathways for removal from aquatic environments (Khetan and Collins, 2007).

Generally, pharmaceuticals are developed in such a manner to limit biodegradation, thereby largely eliminating one of the major pathways. This is because, pharmaceuticals, which are usually designed for oral intake, are as a rule resistant to hydrolysis. The ability of the chemical marker to interact with solid particles, both natural (soils, clay, sediments, microorganisms) or added to the medium (active carbon coagulants), influences the efficiency of pollutant removal from water to a considerable degree. Compounds with low adsorption coefficients tend to remain in the aqueous phase, which favours their mobility through the WWTP or infiltrations through the land mass to the receiving environment (Ohlenbusch et al., 2000; Carballa et al., 2004). Therefore, many pharmaceuticals, such as anti-inflammatories and antibiotics that remain in the aqueous phase are suitable as chemical indicators of sewage or manure contamination, whilst others, such as musks and oestrogens, which are readily adsorbed to solid particles, are unsuitable for this application (Carballa et al., 2004).

Photodegradation is another pathway that can play an important role in the fates and transport of pharmaceuticals to the environment. Many pharmaceuticals contain a number of aromatic rings, heteroatoms and other functional groups that function in direct and indirect photolysis and photochemical processes (Khetan and Collins, 2007; Calisto et al., 2011). This effectively removes the pharmaceutical from the

aquatic environment, although it may result in the formation of persistent by-products, which may in turn be used themselves as chemical markers (Fatta-Kassinos et al., 2011).

Further details on the fate, transport and persistence of pharmaceuticals are given in a number of other review papers (Halling-Sørensen et al., 1998; Sarmah et al., 2006; Khetan and Collins, 2007).

4.3. Occurrence of pharmaceuticals within surface waters

Understanding the occurrence of chemical markers is essential in determining their applicability in forming part of a suite for differentiating sewage and manure. Their occurrence depends upon the use of the particular pharmaceuticals in treatment (Section 4.1) and their fate within the aquatic environment (Section 4.2). In addition, the volume of the receiving water body is another aspect to be considered (Petrovic et al., 2008) since this affects the degree of dilution.

Occurrence characteristics that are of importance in relation to the use of a suite of chemical markers for

differentiating sewage and manure are the concentrations at which the marker occurs and the detection frequency. Within surface waters, pharmaceuticals are commonly detected at concentrations reaching several ng L⁻¹ downstream of WWTP discharges, although concentrations in the µg L⁻¹ range are known (Cahill et al., 2004). Some of the highest reported concentrations of pharmaceuticals and related compounds detected within surface waters are given in Table 2.

With respect to detection frequencies, in several studies, numerous chemical markers have been detected within 100% of samples analysed, e.g. acetaminophen (Kasprzyk-Hordern et al., 2008), atenolol (Kasprzyk-Hordern et al., 2008; Huerta-Fontela et al., 2011), caffeine (Rabiet et al., 2006; Kim et al., 2007; Baker and Kasprzyk-Hordern, 2011), carbamazepine (Glassmeyer et al., 2005; Hao et al., 2008; Kasprzyk-Hordern et al., 2008; Huerta-Fontela et al., 2011), naproxen (Ternes, 1998; Hao et al., 2008; Kasprzyk-Hordern et al., 2008) and lincomycin (Hao et al., 2008; Kasprzyk-Hordern et al., 2008). This indicates their ubiquitous presence within surface waters.

Table 2 – Concentrations of some of the pharmaceuticals and related compounds detected at the highest concentrations within surface waters (n = no. of samples, nd = not detected, NA = not applicable/not determined).

Contaminant	Matrix	Country	Max (ng L ⁻¹)	Mean (ng L ⁻¹)	Median (ng L ⁻¹)	Reference
Propetamphos	Surface water	UK	19.2 × 10 ⁶			(Boxall et al., 2002)
Cypermethrin	Surface water	UK	85100			(Boxall et al., 2002)
Diazinon	Surface water	UK	58000			(Boxall et al., 2002)
Chlorfenvinphos	Surface water	UK	30800			(Boxall et al., 2002)
Lincomycin	Surface water: Following treatment (n = 4)	UK	21100			(Boxall et al., 2006)
Acetaminophen	Streams	US	10000			(Kolpin et al., 2002)
Tramadol	Surface Waters (Downstream of WWTP)	Wales	5970	3522		(Kasprzyk-Hordern et al., 2008)
Ibuprofen	Surface Water (Downstream of WWTP) (n = 5)	UK	5044	1105	826	(Hilton et al., 2003)
Sulfamethazine	River Water (n = 18)	China	4660		100	(Wei et al., 2011)
Oxytetracycline	Surface Water: Following treatment (n = 4)	UK	4490			(Boxall et al., 2006)
Diclofenac	Surface Water (n = 3)	Pakistan	4400		1000	(Scheurell et al., 2009)
Sulfadiazine	Surface Water: Following treatment (n = 4)	UK	4130			(Boxall et al., 2006)
Salicylic Acid	Rivers (n = 43)	Germany	4100		25	(Ternes, 1998)
Sarafloxacin	Streams/River near poultry farm (n = 8)	US	4000			(Campagnolo et al., 2002)
Tramadol	Surface Waters (Upstream of WWTP)	Wales	3468			(Kasprzyk-Hordern et al., 2008)
Bezafibrate	Rivers (n = 43)	Germany	3100	956		(Ternes, 1998)
Bisoprolol	Rivers (n = 43)	Germany	2900		350	(Ternes, 1998)
Caffeine	Surface Water (Downstream 1 of WWTP)	US	2600		Nd	(Glassmeyer et al., 2005)
Chlortetracycline	River Water (n = 18)	China	2420		41	(Wei et al., 2011)
Ibuprofen	Surface Waters (n = 18)	UK	2370		320	(Roberts and Thomas, 2006)
Oxytetracycline	River Water (n = 18)	China	2200			(Wei et al., 2011)
Metoprolol	Rivers (n = 43)	Germany	2200		220	(Ternes, 1998)
Flumethrin	Surface Water	UK	2190		45	(Boxall et al., 2002)
Propetamphos	Surface Water (River) (n = 4)	Poland	2108			(Kasprzyk-Hordern et al., 2007)
Cypermethrin	Streams/River near poultry farm (n = 8)	US	2000			(Campagnolo et al., 2002)

4.4. Pharmaceuticals as sewage and manure chemical markers

In using pharmaceuticals as sewage and manure chemical markers, a combination of a high detection frequency following a sewage or manure input, as applicable, and a low detection frequency where the particular input is absent is necessary in determining the suitability of a particular pharmaceutical as a chemical marker for the differentiation of sewage and manure inputs.

Numerous compounds have been determined to be suitable as chemical markers of sewage contamination. Caffeine has been one of the most studied chemical tracers of sewage to date (Seiler et al., 1999; Picos and de la Cruz, 2000; Chen et al., 2002; Buerge et al., 2003, 2006a, 2006b; Weigel et al., 2004; Peeler et al., 2006; Young et al., 2008; Bartelt-Hunt et al., 2009; Gourmelon et al., 2010). Other pharmaceuticals that have been identified as being suitable as indicators of sewage contamination are shown in Table 3. Of note is that most studies only investigated a small number of pharmaceuticals for their applicability as chemical markers for sewage contamination. Hence, compounds which have

been investigated a larger number of times more likely to be suggested.

In using pharmaceuticals as sewage markers, the level of biodegradability or removal rates within WWTPs is an important consideration. This is because it allows for the distinction between contamination of water with treated and raw sewage (Kasprzyk-Hordern et al., 2009). Therefore, it could provide a more comprehensive characterisation of the input, including information on the type of sewage (raw or treated) discharged to the studied water body (Kasprzyk-Hordern et al., 2009).

Compounds that are susceptible to removal in WWTPs include acetaminophen, ibuprofen, ketoprofen, naproxen, atenolol, gabapentin, caffeine and triclosan (Richardson and Bowron, 1985; Glassmeyer et al., 2005; Kasprzyk-Hordern et al., 2009). Hence, their presence in water bodies should be a direct consequence of raw sewage inputs e.g. spillage of sewage, leaking sewage pipes and septic tanks, sewer overflows and illegal discharge from households (Kasprzyk-Hordern et al., 2009), since they would not be present within treated effluents.

Other compounds that are not removed during wastewater treatment, include carbamazepine, diclofenac, mefenamic acid, codeine, cotinine, diphenhydramine, tramadol, diltiazem, propranolol, cimetidine and sulfasalazine (Anderson et al., 2004; Buerge et al., 2008; Bartelt-Hunt et al., 2009; Kasprzyk-Hordern et al., 2009). This factor makes them good indicators of treated sewage.

Of the compounds suggested as indicators of sewage, ketoprofen (IMB, 2010b; NOAA, 2011) and cimetidine (IMB, 2010b; VMD, 2010; NOAA, 2011) are used in veterinary treatment in addition to human treatment and, hence, are unsuitable as indicators of sewage contamination. The remaining pharmaceuticals have all been detected within surface waters at different concentrations.

In relation to using veterinary pharmaceuticals as markers for manure, seasonal variations are of importance. It has been reported that veterinary pharmaceuticals are more frequently detected in surface runoff during the non-growing season (October to April) than during the growing season (May to September) (Song et al., 2010). Pharmaceuticals resulting from postharvest manure application also appear to be more persistent than from spring application (Song et al., 2010).

The use of antibiotic growth promoters was banned in Europe in 2006 through regulation No. 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition (Kemper, 2008). This has led to a decline in the use of antibiotics such as erythromycin, virginiamycin, chlortetracycline, bacitracin, tylosin, oxytetracycline, sulfathiazole, sulfamethazine, penicillin, lincomycin and apramycin in agriculture (Kemper, 2008). Nevertheless, such pharmaceuticals are still found within European surface waters, indicating their high usage in livestock treatment.

In Table 4, one may note detection frequencies of 100% where other values are given as 'nd', for example gabapentin and tramadol. The reason for this is that not all studies gave data related to the detection frequencies from which the mean detection frequency has been calculated.

Table 3 – Pharmaceuticals suggested (S) as being suitable as indicators of sewage contamination by different studies. 1: (Kasprzyk-Hordern et al., 2009), 2: (Glassmeyer et al., 2005), 3: (Nakada et al., 2008), 4: (Benotti and Brownawell, 2007), 5: (Kolpin et al., 2004), 6: (Young et al., 2008), (Buerge et al., 2006a; Buerge et al., 2008).

Pharmaceutical	Study						
	1	2	3	4	5	6	7
Caffeine	S	S	S	S	S	S	S
Carbamazepine	S	S	S	S	S		
Cotinine				S	S		S
Codeine	S	S					
Gabapentin	S	S					
Ibuprofen	S		S				
Nicotine				S			S
Acetaminophen	S			S			
Triclosan			S			S	
Atenolol	S						
Cimetidine	S						
Crotamiton			S				
Diclofenac	S						
Diltiazem	S						
Diphenylhydramine		S					
Erythromycin H2O	S						
Ethyl citrate		S					
Galaxolide HHCB		S					
Ketoprofen			S				
Mefenamic Acid			S				
Metoprolol	S						
Naproxen			S				
Paraxanthine				S			
Propanolol	S						
Sulfamethoxazole				S			
Sulfasalazine	S						
Thymol			S				
Tonalide AHTN		S					
Tramadol	S						
Valsartan	S						

Table 4 – Summary table for concentrations of the potential chemical markers of sewage contamination within fresh surface waters (nd = not detected, mean detection frequency = mean value of the detection frequency of the contaminant from different studies).

Contaminant	Min (ng L ⁻¹)	Max (ng L ⁻¹)	Mean (ng L ⁻¹)	Mean detection frequency (%)	Reference
Acetaminophen	nd-305	nd-10000	nd-706	59	(Ternes, 1998; Kolpin et al., 2002; Glassmeyer et al., 2005; Gros et al., 2006; Rabiet et al., 2006; Kasprzyk-Hordern et al., 2007; Kim et al., 2007; Pedrouzo et al., 2007; Batt et al., 2008; Conley et al., 2008; Furlong et al., 2008; Kasprzyk-Hordern et al., 2008; Kleywegt et al., 2011)
Atenolol	nd-139.9	22–900	6–470	96	(Zuccato et al., 2000; Calamari et al., 2003; Bendz et al., 2005; Zuccato et al., 2005; Gros et al., 2006; Vanderford and Snyder, 2006; Kasprzyk-Hordern et al., 2007; Batt et al., 2008; Kasprzyk-Hordern et al., 2008; Huerta-Fontela et al., 2011)
Caffeine	nd-421	32.3–2600	13–109	78	(Standley et al., 2000; Buerge et al., 2003; Bendz et al., 2005; Glassmeyer et al., 2005; Peeler et al., 2006; Rabiet et al., 2006; Kim et al., 2007; Pedrouzo et al., 2007; Conley et al., 2008; Furlong et al., 2008; Camacho-Muñoz et al., 2009; Writer et al., 2010; Baker and Kasprzyk-Hordern, 2011; Martínez Bueno et al., 2011)
Carbamazepine	nd-100	4.58–1100	nd-240.8	81	(Ternes, 1998; Öllers et al., 2001; Metcalfe et al., 2003; Wiegel et al., 2004; Bendz et al., 2005; Glassmeyer et al., 2005; Gros et al., 2006; Hao et al., 2006; Rabiet et al., 2006; Vanderford and Snyder, 2006; Kasprzyk-Hordern et al., 2007; Kim et al., 2007; Pedrouzo et al., 2007; Batt et al., 2008; Conley et al., 2008; Furlong et al., 2008; Kasprzyk-Hordern et al., 2008; Camacho-Muñoz et al., 2009; Huerta-Fontela et al., 2011; Kleywegt et al., 2011)
Codeine	nd-170	15–1000	5.4–347	85	(Kolpin et al., 2002; Glassmeyer et al., 2005; Gros et al., 2006; Kasprzyk-Hordern et al., 2007; Furlong et al., 2008; Kasprzyk-Hordern et al., 2008; Baker and Kasprzyk-Hordern, 2011)
Cotinine	nd-23	nd-481	nd-10.4	72.5	(Glassmeyer et al., 2005; Buerge et al., 2008; Furlong et al., 2008; Baker and Kasprzyk-Hordern, 2011; Martínez Bueno et al., 2011)
Diclofenac	nd-700	nd-4400	nd-154	63.4	(Ternes, 1998; Stumpf et al., 1999; Öllers et al., 2001; Hilton et al., 2003; Marchese et al., 2003; Ashton et al., 2004; Wiegel et al., 2004; Bendz et al., 2005; Gros et al., 2006; Rabiet et al., 2006; Vanderford and Snyder, 2006; Kim et al., 2007; Pedrouzo et al., 2007; Zhang and Zhou, 2007; Kasprzyk-Hordern et al., 2008; Camacho-Muñoz et al., 2009; Scheurell et al., 2009)
Diltiazem	nd-139.9	nd-67	nd-19	56	(Kolpin et al., 2002; Glassmeyer et al., 2005; Kasprzyk-Hordern et al., 2007; Batt et al., 2008; Furlong et al., 2008; Kasprzyk-Hordern et al., 2008; Huerta-Fontela et al., 2011)
Diphenhydramine	No data	154–244	nd-11.1	41	(Glassmeyer et al., 2005; Furlong et al., 2008)
Gabapentin	nd-606	75–1879	105–1224	100	(Kasprzyk-Hordern et al., 2007; Kasprzyk-Hordern et al., 2008)
Ibuprofen	nd-144	nd-5044	17–1105	69	(Ternes, 1998; Stumpf et al., 1999; Öllers et al., 2001; Kolpin et al., 2002; Boyd et al., 2003; Calamari et al., 2003; Hilton et al., 2003; Marchese et al., 2003; Metcalfe et al., 2003; Ashton et al., 2004; Bendz et al., 2005; Gros et al., 2006; Roberts and Thomas, 2006; Pedrouzo et al., 2007; Batt et al., 2008; Hao et al., 2008; Kasprzyk-Hordern et al., 2008; Kim et al., 2008; Camacho-Muñoz et al., 2009; Kleywegt et al., 2011)
Meclofenamic Acid	No data	nd	nd	0	(Ternes, 1998; Zhang and Zhou, 2007)

(continued on next page)

Table 4 – (continued)

Contaminant	Min (ng L ⁻¹)	Max (ng L ⁻¹)	Mean (ng L ⁻¹)	Mean detection frequency (%)	Reference
Naproxen	nd-90	nd-551	nd-156.3	76.1	(Ternes, 1998; Stumpf et al., 1999; Öllers et al., 2001; Boyd et al., 2003; Marchese et al., 2003; Metcalfe et al., 2003; Bendz et al., 2005; Gros et al., 2006; Hao et al., 2006; Rabiet et al., 2006; Vanderford and Snyder, 2006; Kim et al., 2007; Pedrouzo et al., 2007; Kasprzyk-Hordern et al., 2008; Camacho-Muñoz et al., 2009; Kleywegt et al., 2011)
Propranolol	nd-35	nd-590	2–54	66	(Ternes, 1998; Hilton et al., 2003; Ashton et al., 2004; Bendz et al., 2005; Roberts and Thomas, 2006; Kasprzyk-Hordern et al., 2007; Pedrouzo et al., 2007; Zhang and Zhou, 2007; Batt et al., 2008; Kasprzyk-Hordern et al., 2008; Camacho-Muñoz et al., 2009; Gros et al., 2009; Huerta-Fontela et al., 2011)
Sulfasalazine	nd-37	16–168	8–66	91	(Kasprzyk-Hordern et al., 2008)
Tramadol	nd-1302	252–5970	227–3522	100	(Kasprzyk-Hordern et al., 2007; Kasprzyk-Hordern et al., 2008; Baker and Kasprzyk-Hordern, 2011)
Triclosan	nd-1.4	nd-74	nd-13	100	(Lindstrom et al., 2002; Boyd et al., 2003; Vanderford and Snyder, 2006)

In relation to using veterinary pharmaceuticals as markers for manure, seasonal variations seem to be of importance. It has been reported that veterinary pharmaceuticals are more frequently detected in surface runoff during the non-growing season (October to April) than during the growing season (May to September) (Song et al., 2010). Pharmaceuticals resulting from postharvest manure application also appear to be more persistent than from spring application (Song et al., 2010).

The use of antibiotic growth promoters was banned in Europe in 2006 through regulation No. 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition (Kemper, 2008). This has led to a decline in the use of antibiotics such as erythromycin, virginiamycin, chlortetracycline, bacitracin, tylosin, oxytetracycline, sulfathiazole, sulfamethazine, penicillin, lincomycin and apramycin in agriculture (Kemper, 2008). Nevertheless, such pharmaceuticals are still found within European surface waters, indicating their high usage in livestock treatment.

No study is known where the suitability of veterinary pharmaceuticals as markers of manure contamination was specifically investigated. However, studies on human pharmaceuticals indicate that the occurrence of pharmaceuticals within the aquatic environment is directly related to their use within the community being studied, their metabolic degradation and environmental degradation. A number of studies have determined lists of pharmaceuticals that are, for example, important in animal medicine (Kemper, 2008), have a high potential to reach the environment and are commonly used (Capleton et al., 2006) or of potential concern in relation to the aquatic environment (Boxall et al., 2003). Hence, this data may be utilised in determining the suite of chemical markers through understanding the veterinary pharmaceuticals that have the highest potential to act as chemical markers.

In order to use a veterinary pharmaceutical as a chemical marker to differentiate sewage and manure as a source of

nitrate contamination, it must not be used in human treatment. Alternatively, their usage within veterinary treatment should be extensively higher than usage in human treatment and vice versa. For example, compounds such as trimethoprim, tetracycline, amoxicillin, erythromycin, ampicillin and neomycin are approved for both human and veterinary use and, therefore, they would not be useful in this capacity (IMB, 2010a; IMB, 2010b; VMD, 2010).

The concentrations of various veterinary pharmaceuticals, which fulfil the criteria of being used only within veterinary treatment and having been detected within surface waters as a result of their high usage rates, is given in Table 5. Of note is that a very small proportion of these studies were carried out on sites that are potentially affected by manure, since most have focused on the occurrence of pharmaceuticals within surface waters influenced by WWTP effluents. Such a factor is likely to be a contributing factor to the low concentrations and detection frequencies of the compounds.

4.5. Considerations for the suite of chemical markers

The determination of the suite of chemical markers is a critical factor for such an environmental forensics application as the differentiation of sewage and manure. The major considerations of importance have been identified to be marker specificity and detection frequency.

In relation to marker specificity, the chemical markers selected, must, in the first instance, be specific to human or veterinary treatment therefore allowing for differentiation between sewage and manure. Other considerations, such as environmental fates and transformations and specific uses (e.g. used to treat a specific group of animals or only used in hospital treatments) would allow for further characterisation of the nitrate source as being e.g. raw or treated sewage, or emanating from a particular type of manure,

Table 5 – Summary table for concentrations (ng L⁻¹) of the potential chemical markers of manure contamination within fresh surface waters (nd = not detected, NA = not applicable/not determined).

Contaminant	Max (ng L ⁻¹)	Mean (ng L ⁻¹)	Mean detection frequency (%)	Reference
Chlortetracycline	nd-2420	NA	44	(Boxall et al., 2002; Campagnolo et al., 2002; Lindsey et al., 2003; Arikan et al., 2008; Gros et al., 2009; Wei et al., 2011)
Cypermethrin	85100	NA	NA	(Boxall et al., 2002)
Diazinon	58000	NA	NA	(Boxall et al., 2002)
Doramectin	nd	NA	NA	(Boxall et al., 2006)
Emamectin	nd	NA	NA	(Boxall et al., 2002)
Enrofloxacin	nd-13	NA	2	(Campagnolo et al., 2002; Gros et al., 2009; Kleywegt et al., 2011)
Flumethrin	2190	NA	NA	(Boxall et al., 2002)
Ivermectin	nd	NA	NA	(Boxall et al., 2006)
Lincomycin	4.6–21100	8.2–26.6	59.5	(Zuccato et al., 2000; Campagnolo et al., 2002; Kolpin et al., 2002; Calamari et al., 2003; Zuccato et al., 2005; Boxall et al., 2006; Brown et al., 2006; Hao et al., 2006; Kleywegt et al., 2011)
Monensin	37–810	nd-104.7	38.5	(Hao et al., 2006; Song et al., 2007; Zhang and Zhou, 2007; Kleywegt et al., 2011)
Norfloxacin	nd-120	8	2	(Campagnolo et al., 2002; Kolpin et al., 2002; Brown et al., 2006; Gros et al., 2009; Kleywegt et al., 2011)
Propetamphos	19.2 × 10 ⁶	NA	NA	(Boxall et al., 2002)
Sarafloxacin	4000	NA	NA	(Campagnolo et al., 2002)
Sulfadiazine	9–4130	6	55	(Boxall et al., 2006; Perret et al., 2006; Gros et al., 2009; García-Galán et al., 2011; Wei et al., 2011)
Sulfadimethoxine	3–1500	NA	NA	(Lindsey et al., 2001; Campagnolo et al., 2002; Perret et al., 2006; Arikan et al., 2008; García-Galán et al., 2011)
Tilmicosin	nd-0.43	NA	NA	(Zuccato et al., 2000; Calamari et al., 2003; Gros et al., 2009)
Tylosin	nd-39	9.8	32.4	(Zuccato et al., 2000; Calamari et al., 2003; Brown et al., 2006; Song et al., 2007; Hao et al., 2008; Gros et al., 2009; Kleywegt et al., 2011)

therefore leading to further characterisation of the nitrate source.

A high detection frequency is another necessity for ideal chemical markers as part of the suite developed. The usefulness of a particular marker decreases if it is infrequently detected downstream of its particular source, since this would require a much larger complement of markers within the suite for conclusive source differentiation. In addition, the occurrence of a particular chemical marker at higher concentrations facilitates its detection particularly when taking into account the complex matrices that are commonly investigated in relation to trace contamination within surface waters.

The use of a smaller suite of chemical markers furthermore reduces method development complexity and resulting in a shorter analysis time. This could be achieved through an understanding of the persistence of the chemical markers (a persistent chemical marker is more likely to have a high detection frequency) and the usage levels within the area being investigated, through an investigation of the prescription and sales levels of the different chemical markers within

the community being studied. Studies on consumption patterns and volumes in the water body's catchment area are critical and, if they differ widely, can result in studies carried out in one area not being applicable in another setting (Petrovic et al., 2008; Kümmerer, 2009). This is because the pharmaceuticals that are most frequently detected represent those that are dispensed at the highest levels in the community (Kasprzyk-Hordern et al., 2008).

Another important factor is that a developed suite of chemical markers must be periodically reviewed and possibly modified. This is largely due to changes in usage characteristics, which may have a great impact on the detection frequency. For example, Andreozzi et al. (2003) reported that clofibric acid (the major metabolite of clofibrate, etofibrate and etofyllinclofibrate), which was previously reported as one of the most common pharmaceutical residues in effluents from WWTPs and in natural waters in Germany (Ternes, 1998), was only detected in about half of the studied effluents. This has been attributed to the drugs that are metabolised to clofibric acid being replaced with others, such as

gemfibrozil and fenofibrate, within the studied communities (Andreozzi et al., 2003).

Finally, environmental concerns related to pharmaceutical use are ever increasing. Nevertheless, the reduction of drug inputs into the environment through restricting or banning their use is not perceived to be feasible due to their beneficial health effects and economic importance (Khetan and Collins, 2007; Petrovic et al., 2008). Moreover, pharmaceutical use is expected to grow with the increasing age of the population (Petrovic et al., 2008). This makes pharmaceuticals well positioned for use as chemical markers of sewage and manure contamination.

5. Conclusions

Nitrate source determination is considered as an important factor for improving our health and environment, and it has a legislative importance in relation to the Water Framework Directive. However, comprehensive studies into means to achieve nitrate source determination are limited. Most investigations look into a single method for nitrate source determination resulting in a limited scope of application. It is proposed in this paper that the use of a suite of isotopic and chemical markers is suitable for differentiating between the major sources of contamination within water bodies.

The dual isotopic approach is accepted as a method of discriminating between most nitrate sources. Through the inclusion of chemical marker indicators, such as pharmaceuticals and food additives, the specific differentiation between sewage and manure may be achieved, thereby filling the gap in knowledge provided through the use of isotopic markers in isolation for characterising nitrate sources. The use of a suite of chemical markers consisting of labile and conservative chemical markers for the identification of raw and treated sewage inputs, as well as veterinary pharmaceuticals used for treating different animals has been suggested, and a list of chemical markers with such criteria identified. However, further research in this as yet little explored field is necessary to confirm the suitability of this suite of markers for such an application, and is therefore the focus of research within our research group. In particular, further studies into the use of veterinary-linked chemical markers are necessary.

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Appendix. Supplementary data

Supplementary data related to this article can be found online at [doi:10.1016/j.watres.2012.01.044](https://doi.org/10.1016/j.watres.2012.01.044).

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