Thomas Hofstetter, environmental chemist, is a member of the Environmental Chemistry department and senior lecturer at the ETH Zurich.
Co-authors: Reto Wijker, Stephanie Spahr

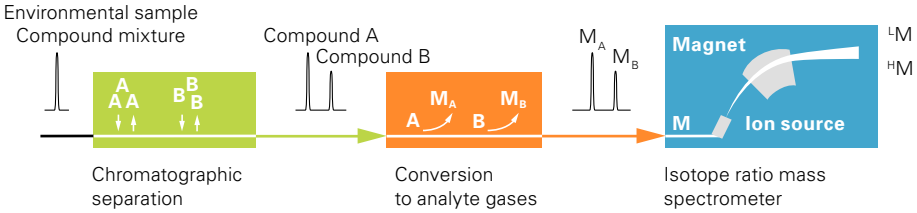
Isotopes: tracking pollutant sources and breakdown

The fate of organic micropollutants in soils and natural waters is difficult to track using traditional methods. But with compound-specific isotope analysis, the isotopic composition of contaminants can be studied. This makes it possible, for example, to trace the origin of dishwasher detergents or to determine the degradation pathways of explosives.

Reto Wijker

Remediation of a contaminated site formerly used by a Swiss explosives manufacturer: with the aid of isotope analysis, degradation processes in the subsurface can be reconstructed.

In assessing the quality of natural waters, it is essential to take organic micropollutants into account [1]. Tens of thousands of chemicals are used in industry, commerce and households. In spite of wastewater treatment efforts, many of these substances end up in surface waters, where they can cause adverse effects even at low concentrations. In addition, organic contaminants are released from landfills and contaminated sites. As concentrations of these substances often exceed those in



Schematic view of compound-specific isotope analysis (adapted from [2]). After being separated by chromatography, individual compounds are converted into a measurement gas (M) suitable for analysis. An isotope ratio mass spectrometer simultaneously measures two ion streams of light (abundant, ^{L}M) and heavy (rare, ^{H}M) isotopologues.

soils and waters, contaminant immobilization or site remediation measures need to be considered. In deciding whether action is required in practice, the following questions are particularly relevant: Where did the pollutants originate, and who might be responsible for their release into the environment? If transformation processes occur, do they lead to toxic or benign products?

More accurate picture of sources and degradation

With modern analytical methods such as liquid chromatography coupled to high-resolution mass spectrometry (see the article on page 6), trace organic contaminants can be identified and their concentrations quantified. However, identifying the source becomes challenging if various emitters could be responsible. And decreasing concentrations do not necessarily indicate transformation, as such observations may be due to dilution, sorption of micropollutants to particles and sediments, volatilization or degradation. Determining if and to what extent contaminants are typically degraded requires labour-intensive measurements supported by modelling efforts. In addition, transformation products may also be problematic in terms of ecotoxicity and should be included in risk assessment.

Using compound-specific isotope analysis, researchers at Eawag are developing new approaches for determining the source of organic contaminants and characterizing transformation processes. With compound-specific isotope analysis, stable isotope ratios can be measured for individual compounds – typically carbon ($^{13}C/^{12}C$), nitrogen ($^{15}N/^{14}N$) and hydrogen ($^{2}H/^{1}H$) (see box). First, however, the compounds in an environmental sample need to be separated chromatographically from other constituents. This is generally done by gas chromatography coupled to isotope ratio mass spectrometry. Intensive research efforts are currently under way to expand the range of applicable separation methods (e.g. liquid chromatography) and elements amenable to isotope analysis, such as chlorine ($^{37}Cl/^{35}Cl$) and bromine ($^{81}Br/^{79}Br$) [2].

To illustrate the application of compound-specific isotope analysis (CSIA), we discuss two examples of recent Eawag research.

Compound-specific stable isotope analysis

Organic compounds contain a variety of isotopic elements and thus numerous isotopologues (molecules differing only in their isotopic composition). It is therefore not possible to determine isotope ratios directly from the molecular mass with sufficient precision using conventional mass spectrometry.

In **compound-specific isotope analysis** (CSIA), to solve this problem, each compound is converted into a measurement gas of low mass, in which only one or two atoms of the element to be analysed (analyte) are present. In an isotope ratio mass spectrometer (IRMS), the isotopes of the analyte can be simultaneously measured as two separate ion streams, containing the heavy (rare) and the light (abundant) isotopologue. For example, the carbon skeleton of a contaminant is oxidized to CO_2 at around 1000 °C and quantified in the mass spectrometer as the ratio of $^{13}CO_2$ to $^{12}CO_2$. To analyse nitrogen, hydrogen and oxygen isotopes, N_2, H_2 and CO are used as the analyte gases.

The measurement of an isotope ratio carried out in this way is extremely precise even if the abundances of the isotopes differ markedly (^{13}C makes up only 1.1 % of the element carbon, and ^{2}H only 0.015 % of hydrogen). However, the method is only applicable for elements which can be continuously converted to analyte gases.

In addition, because isotope measurements vary from one instrument to another, measured isotope ratios always have to be normalized to standard reference materials. The result is the **isotope signature** given as a δ value, which expresses the per mil (‰) difference between the isotope ratio in the sample and that in the reference material. In the case of carbon isotope signatures (δ13C), for example, the following equation applies:

$$\delta^{13}C\ (‰) = \frac{^{13}C/^{12}C\ (\text{sample})}{^{13}C/^{12}C\ (\text{reference})} - 1$$

With gas chromatography coupled to isotope ratio mass spectrometry, the typical measurement uncertainty for carbon isotopes is ±0.5‰. For two measurements, this corresponds to a difference in the $^{13}C/^{12}C$ isotope ratio of only 0.0000056.

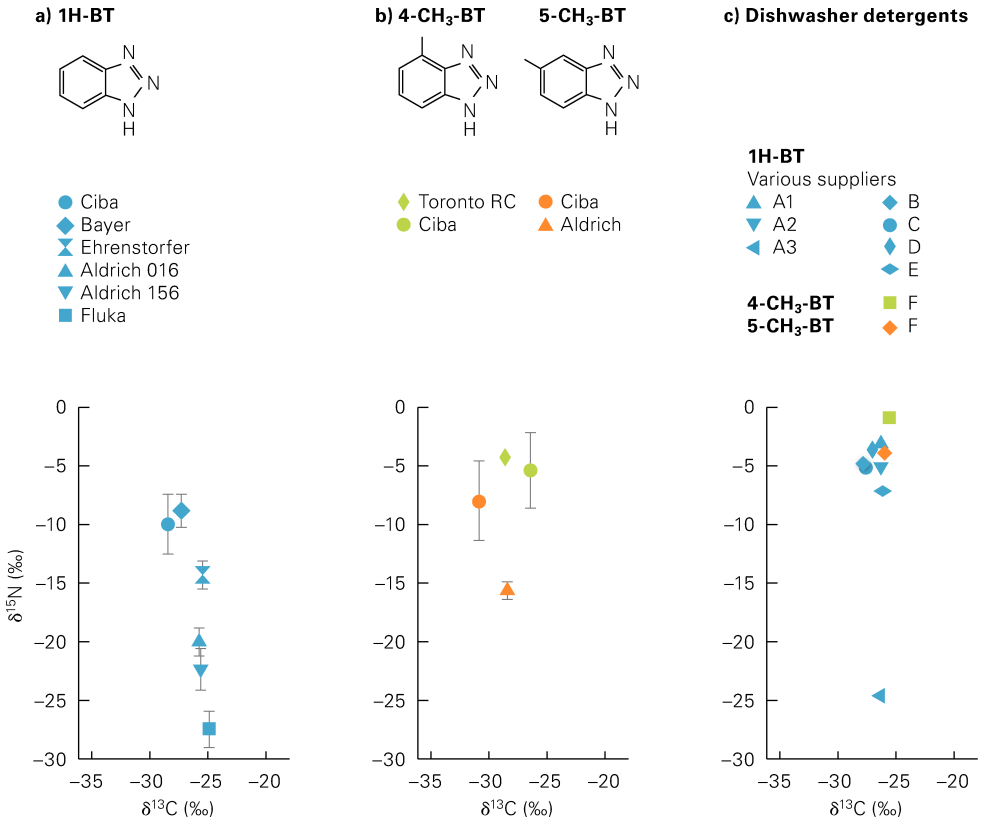


Fig. 1: Carbon and nitrogen isotope signatures of benzotriazole (1H-BT) and two methylbenzotriazoles (4-CH_3-BT, 5-CH_3-BT) in chemicals from various suppliers. Shown on the right are the $\delta^{13}C$ and $\delta^{15}N$ values for the same substances in dishwasher detergents from various suppliers (adapted from [6]).

As is shown for benzotriazoles, similar isotope ratios are indicative of identical production processes and raw materials, thus permitting identification of the source. Varying isotope ratios, however, as observed for nitroaromatic compounds at a site contaminated with explosives, provide evidence of transformation processes and products, and also – with additional information – rates of degradation[3].

The origin of benzotriazoles in dishwasher detergents

Benzotriazoles are among the most frequently detected organic micropollutants in Swiss waters because they are used in large quantities in industrial and consumer products such as corrosion inhibitors and antifreeze agents [4]. They serve as indicators of persistent organic pollutants and are used to assess the performance of wastewater treatment plants (WWTPs) [5]. Whether and under what conditions benzotriazoles (and presumably also other micropollutants) in surface waters can be biodegraded at WWTPs could be determined with the aid of CSIA. This would require the detection of changes in the composition of the carbon or nitrogen isotopes in benzotriazoles. However, because of their tendency to form complexes with metals, benzotriazoles cannot be measured by the conventional method of gas chromatography coupled to isotope ratio mass spectrometry. At Eawag, we have therefore developed an alternative approach for CSIA of such polar organic micropollutants. On this basis, we tentatively assigned benzotriazoles in dishwasher detergents to possible suppliers.

Isotope analysis involves comparison of the very different signal intensities of heavy and light isotopes, and thus requires high contaminant concentrations. Our newly developed approach, using nickel-platinum reactors to convert benzotriazoles to CO_2 and N_2, requires 22 nanograms of the element carbon and 11 nanograms of nitrogen for accurate measurement. In a microlitre injection, this is equivalent to a benzotriazole concentration of 30 – 40 milligrams per litre. While such quantities are relatively low for CSIA, the concentrations are 10,000 times higher than those typically observed in surface waters or WWTP effluents. For this reason, it is necessary to enrich benzotriazoles from environmental samples using solid-phase extraction, without altering the isotopic composition of the target compounds. It is then possible to determine the C and N isotope signatures of benzotriazole (1H-BT) and methylbenzotriazoles (4-CH_3-BT and 5-CH_3-BT) in tap water, WWTP effluents, sewage sludge and dishwasher detergents by gas chromatography coupled to isotope ratio mass spectrometry.

Isotopic fingerprint determined by chemical production

Figures 1a and b show the $\delta^{13}C$ and $\delta^{15}N$ values of benzotriazoles from various chemical suppliers. It is noticeable that the

Remnants of TNT and DNT at the contaminated site.

Reto Wijker

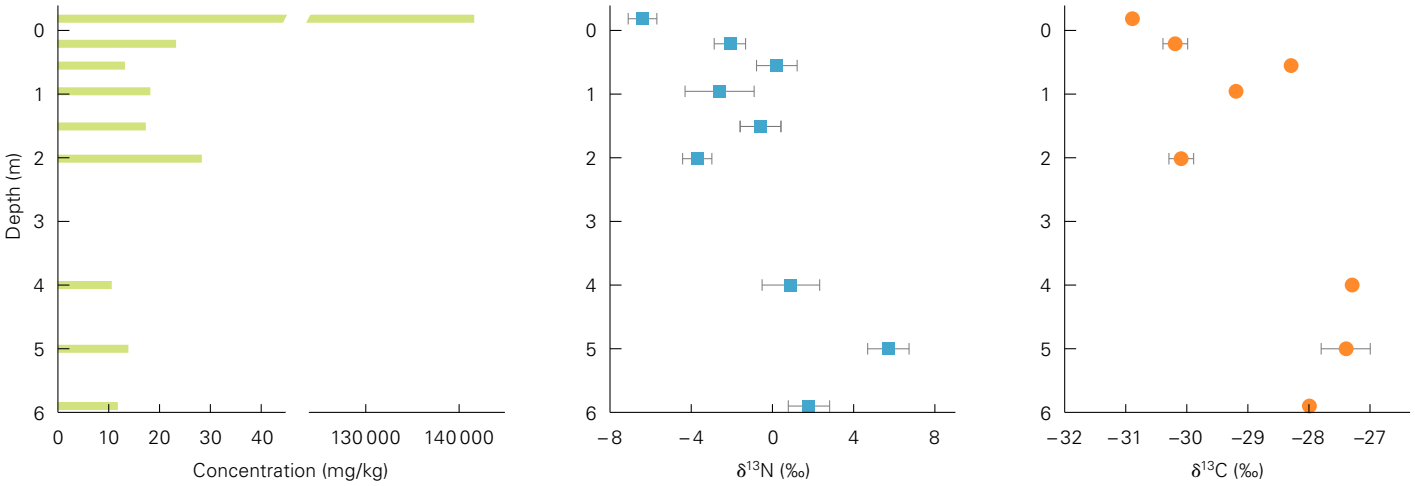


Fig. 2: Concentration profile for the explosive 2,4-DNT over a depth of 6 metres and the carbon and nitrogen isotope signatures (adapted from [7]).

benzotriazoles and methylbenzotriazoles produced by different manufacturers differ mainly in the nitrogen isotope ratios. This can be explained by the routes used for synthesis. While the benzene exhibits $\delta^{13}C$ values between –25 and –30‰, which are typical for petrochemicals, the triazole ring has to be produced from chlorinated nitrobenzene in a series of reaction steps. Moreover, other nitrogen-containing compounds such as ammonia and nitrous acid are also used. The widely varying $\delta^{15}N$ values of the benzotriazoles thus reflect the nitrogen isotope ratios of these precursors. In addition, during the synthesis of industrial chemicals, the isotope ratios of the reactants and products are modified because chemical reactions rarely go to completion. The combination of these two factors – the isotope ratios in the precursors and their modification by chemical reactions – produces the benzotriazoles' isotopic fingerprint.

We also investigated whether the benzotriazoles in dishwasher detergents available in Switzerland originate from different chemical manufacturers (Fig. 1c). We found that, with one exception, the nitrogen isotope signatures are very similar (–5‰), which suggests that most of the benzotriazoles are produced by the same manufacturer. In future studies, we plan to investigate whether benzotriazoles in Swiss rivers reflect the findings for dishwasher detergents. To this end, a new project involving CSIA of micropollutants has been launched by Eawag, in collaboration with the University of Neuchâtel and

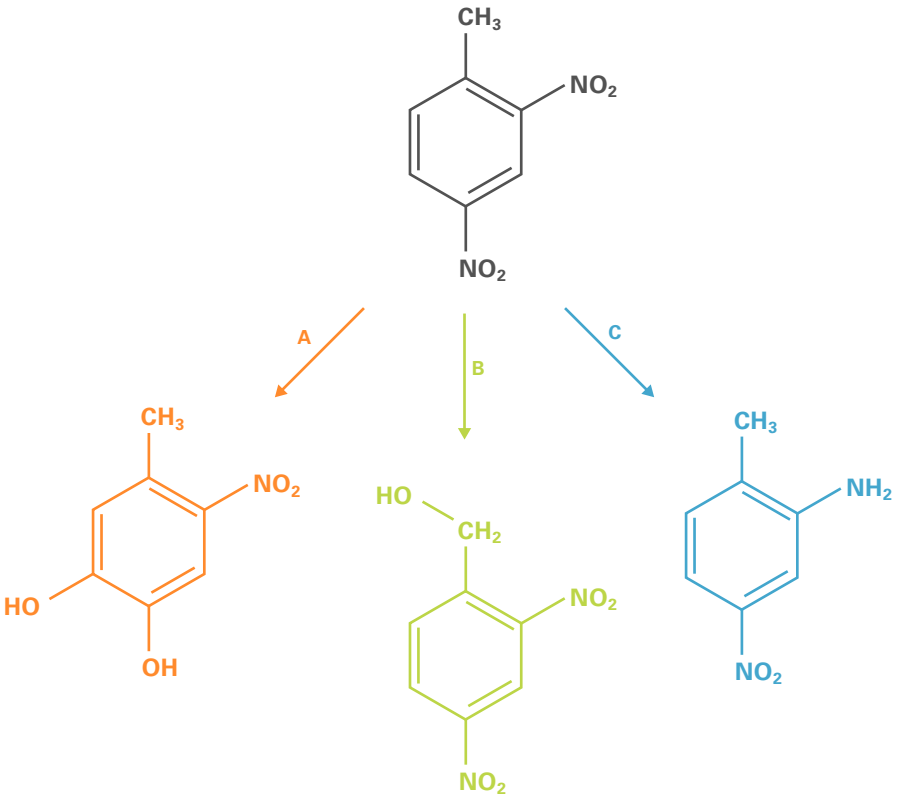


Fig. 3: Initial reaction steps in the microbial biodegradation of 2,4-DNT via dioxygenation (A), methyl-group oxidation (B) and nitro-group reduction (C, only one of two possible products shown). For 2,4-DNT, only dioxygenation leads to mineralization.

the German Research Center for Environmental Health (Helmholtz Zentrum München), with support from the Swiss National Science Foundation.

Reconstructing the degradation of explosives

Nitroaromatic compounds such as the explosive trinitrotoluene (TNT) and its precursor dinitrotoluene (DNT) occur as toxic substances at sites contaminated with explosives or in the polluted subsurface of former production sites. Although their degradation pathways are known, it is extremely difficult to estimate the extent and rate of degradation of this class of substances in the environment. This is because degradation takes place over decades. Secondly, isotopic analysis is complicated by the fact that both the substances and their transformation products are often strongly bound to the organic and mineral matrix of soils or sediments. In addition, many nitroaromatic compounds are transformed via a number of reactions that may give rise to even more toxic products, such as aromatic amines.

At a contaminated site in Switzerland, Eawag has now, for the first time, successfully evaluated the biodegradation of nitroaromatic compounds on the basis of changes in isotope signatures – so-called isotope fractionation [7]. Figure 2 shows a concentration/depth profile for 2,4-DNT at the highly contaminated spot. Comparable data are available for TNT and 2,6-DNT. The concentrations measured in the subsurface indicate transport of the contaminants to the subsurface. Whether degradation also occurs in this process cannot be determined from these data alone. It is known, however, that microorganisms can degrade 2,4-DNT via three different reactions, only one of which leads to mineralization (Fig. 3).

The changes observed in the C and N isotope signatures of 2,4-DNT with increasing depth provide information on microbial degradation. The lower the concentrations of DNT in the subsurface, the more strongly do the $\delta^{13}C$ and $\delta^{15}N$ values differ from soil-surface samples. This observation provides robust evidence of (bio)chemical transformation. Substantial isotope fractionations of this kind cannot be caused by transport processes and require the cleavage of chemical bonds in processes such as microbial degradation. This physicochemical phenomenon is known as a kinetic isotope effect. As kinetic isotope effects vary depending on the element and the type of chemical bonds broken, isotope fractionation can also be used to identify the reaction mechanism and thus the degradation pathway in the environment [8].

When $\delta^{13}C$ and $\delta^{15}N$ values from Figure 2 are plotted against each other, a systematic trend towards heavier carbon and nitrogen isotope signatures can be seen for 2,4-DNT. With the aid of typical isotope fractionation trends observed for 2,4-DNT degradation in the laboratory, this trend can be assigned to specific degradation pathways (Fig. 4). If a contaminant is simultaneously eliminated by different mechanisms, the isotope fractionation trends reflect a combination of the various degradation processes. Accordingly, the isotope signatures observed in the contaminated subsurface show that 2,4-DNT was largely eliminated by dioxygenation, which leads to complete mineralization.

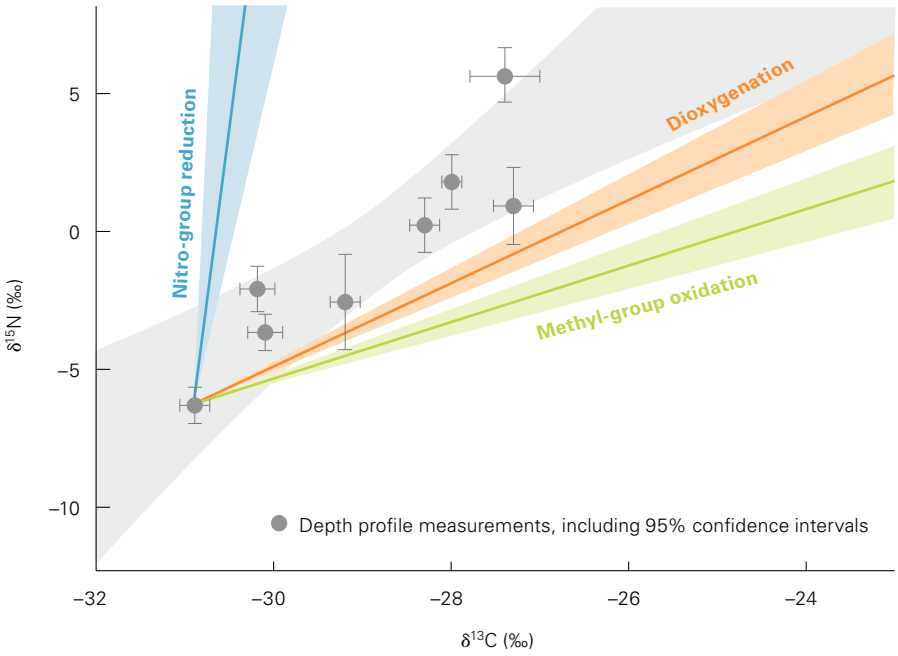


Fig. 4: Isotope fractionation analysis of 2,4-DNT in a contaminated subsurface (grey circles). The enrichment of ^{13}C and ^{15}N in the course of degradation follows trends observed in laboratory experiments for mineralization via dioxygenation (orange trend curve) and for reduction to aminonitrotoluenes (blue trend curve) (adapted from [7]).

The remainder was transformed by nitro-group reduction to problematic aminonitrotoluenes. Compounds of this kind were also detected in our samples.

In order to use the instrument of CSIA in a more quantitative way, we are currently investigating the extent of isotope fractionation and the underlying kinetic isotope effects of various reaction mechanisms responsible for the biodegradation of nitroaromatic compounds. Initial laboratory studies with 2,4-DNT show that, for a given quantity of substance transformed, dioxygenation causes relatively low fractionation of carbon and nitrogen isotopes. As this is the predominant degradation reaction, the high isotope fractionation observed at the field site indicates that a large proportion of this contaminant (over 99 per cent) must have been mineralized. Owing to the high levels of contamination, however, the contaminant was still detectable. If one compares the estimated quantities degraded with historical data for production periods, a half-life of 10–50 years can be calculated for 2,4-DNT in the subsurface. Even if the uncertainty associated with the degradation rate amounts to one or two decades, CSIA provides information on the extent and pathway of contaminant transformation which cannot be obtained by conventional methods.

thomas.hofstetter@eawag.ch

[1] Schwarzenbach R.P., Egli T., Hofstetter T.B., von Gunten U., Wehrli B. (2010): Global water pollution and human health. Annual Review of Environment and Resources *35 (1),* 109–136.
[2] Elsner M., Jochmann M.A., Hofstetter T.B., Hunkeler D., Bernstein A., Schmidt T.C., Schimmelmann A. (2012): Current challenges in compound-specific stable isotope analysis of environmental organic contaminants. Analytical and Bioanalytical Chemistry *403 (9),* 2471–2491.
[3] Hofstetter T.B., Berg M. (2011): Assessing transformation processes of organic contaminants by compound-specific stable isotope analyses. Trends in Analytical Chemistry *30 (4),* 618–627.
[4] Hollender J., Zimmermann S.G., Köpke S., Krauss M., McArdell C.S., Ort C., Singer H., von Gunten U., Siegrist H. (2009): Elimination of organic micropollutants in a municipal wastewater treatment plant upgraded with a full-scale post-ozonation followed by sand filtration. Environmental Science and Technology *43 (20),* 7862–7869.
[5] Götz C. et al. (2010): Mikroverunreinigungen – Beurteilung weitergehender Abwasserreinigungsverfahren anhand Indikatorsubstanzen. GWA *4,* 325–333.
[6] Spahr S., Huntscha S., Bolotin J., Meier M.P., Elsner M., Hollender J., Hofstetter T.B. (2012): Compound-specific isotope analysis of benzotriazole and its derivatives. Analytical and Bioanalytical Chemistry. http://dx.doi.org/10.1007/s00216-012-6526-1
[7] Wijker R.S., Bolotin J., Nishino S.F., Spain J.C., Hofstetter T.B. (in preparation): Evaluating nitroaromatic explosives biodegradation at a contaminated field site using compound-specific C and N isotope analysis. Environmental Science and Technology.
[8] Hofstetter T.B., Schwarzenbach R.P., Bernasconi S.M. (2008): Assessing transformation processes of organic compounds using stable isotope fractionation. Environmental Science and Technology *42 (21),* 7737–7743.