TETRACYCLINE AND METABOLITES IN AGRICULTURAL FARMLAND SOIL AND *PETROSELINUM CRISPUM* VAR. *NEAPOLITANUM* ROOTS AND LEAFS

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Abstract
ELISA screening method (suitable analytical technique with specificity, sensitivity and simplicity) was used to monitories the TCS accumulation in agricultural farmland soil and Garden Parsley (Petroselinum Crispum var. neapolitanum) roots and leafs. Residues of TCs, used in veterinary and human medicine, end up in the agricultural farmlands due to the common practice of land application of manure. These excreted TCs, from treated animals, are quite persistent in soil and can accumulate with repeated manure application and it can lead to an increase of the TCs concentration in cultivated vegetables used as human food. The main goal of this study is to study the TCs accumulation in the soil and cultivated Petroselinum crispum var. neapolitanum-radix and leafs. The samples were taken from an agricultural farmland in NW-Romania.

Key words: tetracycline, ELISA, soil, Parsley

INTRODUCTION

Pharmaceuticals uses for human and veterinary purpose are continuously introduced into the environment. Their impact on the ecosystem is still partly missing. The traces of pharmaceuticals residues can be detected at ng L^{-1} levels because of the instrumental analytical chemistry development. Recent analytical methods are the Immunoassays approach. Those methods show attractive features for pharmaceuticals trace analysis due to the fact that they are inexpensive, require little sample pre-treatment, exhibit high sensitivity and are very easy to manage (Buchberge, 2007). The other methods used for medicine detection as HPLC, GC are time-consuming, expensive, require complex laboratory equipment and trained personnel and require tedious sample-preparation procedures based on solid-phase extraction (SPE) and multi-step clean-up (Chafer-Pericas et al, 2010).

ELISA (enzyme-linked immunosorbent assay) methods is based on an enzyme catalyst and UV/vis detection. It is a technical protocol highlighting the antibodies against viruses and bacteria or specific antibodies against a specific type of virus, so the analysis reveals not the pathogen but determined the enzyme activity that binds to antigen or antibody, thus giving indication of the amount of antagonistic substance produced against it (Font et al, 2008; Zhao et al, 2007; Wang et al, 2009).

Immunoassays (ELISA) technique applied for the pollution with medicine of environmental studies are quite rare because the commercially available kits for pharmaceuticals are in most cases optimized for samples like human fluids or food and also because immunoassays are not suited for simultaneous determination of analytes with different chemical structures. Therefore, in the studies of medicine impact on the environmental it might be useful to select a drug that is practically always present in environmental samples. In this way, the quantisation of just one compound can give information about the extent of contamination by pharmaceuticals (Buchberge, 2007).

A pharmaceutical compound that can function as a marker might be tetracycline, a broad spectrum antibiotic. TCs have been used for over 50 years in human health and in agricultural and veterinary practices, primarily as antibacterial agents. In addition to therapeutic uses, antibiotics are also applied for nontherapeutic reasons as growth promotion in animals, although European Union (EU) legislation has forbidden this practice since 2006 (European Commission 2003). Large amounts of antibiotics continue to be used worldwide in the treatment of humans and animals. The ultimate fate of antibiotics consumed by both humans and animals is a main factor in the potential of their release into the environment. Pharmacokinetic studies have determined that administrated antibiotics are largely excreted from humans and animals in the parent compound form (up to 90%) or as metabolites. This largely depends on the type of antibiotic. Metabolites may conjugate with glucose or other polar compounds and convert back to the original parent compound in the environment with microorganism activity (Sung-Chul, Kenneth, 2007).

Kumar et al. (Kumar et al, 2004) used ELISA kits commercially available for meat and milk samples to determine residues of tylosin and TCs in surface and ground water. A similar ELISA kit was recently applied for detecting oxytetracycline used as an animal feed additive in fish farms (Himmelsbach, Buchberger, 2005). Both water samples and sediment samples have been analyzed. Concentrations down to 1 $g{\cdot}L^{-1}$ for water samples and 1 $g{\cdot}g^{-1}$ for sediment sample could easily be analyzed (considerably lower detection limits seem possible if the ELISA procedure is combined with sample pre concentration). The correlation coefficient for results from the ELISA and from HPLC was 0.98 (calculated for 11 real samples) (Buchberge, 2007*)*.

Residues of TCs end up in the environment due to the common practice of land application of manure from treated animals (Hamscher et al 2005). These excreted TCs are quite persistent in soil and can accumulate with repeated manure application (De et al, 2008), which will ultimately be accumulated in the crops.

The main goal of this study is to pursue in the agricultural farmlands where the manure application is used as fertilizer the TCs accumulation in the soil and cultivated *Petroselinum crispum* var. *neapolitanum* radix and leaves.

Garden parsley is a species of *Petroselinum* in the family *Apiaceae*. In addition to its widespread use (root and leaves) as a garnish, parsley offers numerous health benefits. Parsley is a good source of antioxidants (especially luteolin), folic acid, vitamin C, and vitamin A. Proclaimed health benefits include anti-inflammatory properties and a boosted immune system. It is a bright green, hairless, biennial, herbaceous plant in temperate climates, or annual herb in subtropical and tropical areas. Where it grows as a biennial, in the first year, it forms a rosette of tripinnate leaves 10–25 cm long with numerous 1–3 cm leaflets, and a taproot used as a food store over the winter. In the second year, it grows a flowering stem to 75 cm tall with sparser leaves and flat-topped 3–10 cm diameter umbels with numerous 2 mm diameter yellow to yellowish-green flowers. The seeds are ovoid, 2–3 mm long, with prominent style remnants at the apex. One of the compounds of the essential oil isapiol. The plant normally dies after seed maturation (Blamey, Grey, 1989). Parsley grows best in moist, well drained soil, with full sun. It grows best between 22–30 °C, and is usually grown from seed. Germination is slow, taking four to six weeks and often difficult because of furanocoumarins in its seed coat. Plants grown for the leaf crop are typically spaced 10 cm apart, while those grown as a root crop are typically spaced 20 cm apart to allow for the root development (Huxley, 1992). The two main groups of parsley used as herbs are curly leaf (i.e.) (*P. crispum crispum* group; syn. *P. crispum* var. *crispum*) and Italian, or flat leaf (*P. crispum neapolitanum* group; syn. *P. crispum* var. *neapolitanum*); of these, the neapolitanum group more closely resembles the natural wild species. Flat-leaved parsley is preferred by some as it is easier to cultivate, being more tolerant of both rain and sunshine, and has a stronger flavor (though this is disputed), while curly leaf parsley is preferred by others because of its more decorative appearance in garnishing. A third type, sometimes grown in southern Italy, has thick, celery-like leaf stems (http://en.wikipedia.org/wiki/Parsley).

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MATERIALS AND METHODS

The studied area was a family agricultural farmland (in Sântandrei village) near Oradea City, in NW Romania. The soil of the studied farmland is cultivated with vegetables, each year, in the past 50 years, using only cows and horses manure derived from village livestock. The manure was added in every autumn. The soil contained 51.2% clay, 1.88% organic carbon contents and 14% (pH 6.4). The sampling process was due during the spring, summer and autumn 2012.

The soil samples were taken using Ekman sampler from the surface of land layer 1-10 cm depth in. Sampling was performed from 20 sampling points well establish in the cultivated land (within an area of 100 m×120 m at 10 cm interval below the surface) and 20 nearby in the land that was not cultivated. Those last 20 sampling points were establish after an intensive soil survey of the uncultivated field, subplots were chosen with similar soil properties and profiles that were at least 500 m away from the field border and more than ten years wasted (Fodor et al, 2013).

The studied plant was *P. crispum* var. *neapolitanum.* The studied plant parts were the root and the leaves from plants in second year of development. Therefore it is expected that the two plant parts exhibits different antibiotics accumulation properties.

The cultivated plant samples were taken from each 20 sampling point from the cultivated land. The collected samples were transported to laboratory, were cut in small pieces and dried in liofilizator at -40˚C. The dried samples were homogenized in grinder and kept in dark place until measurements.

Along the study, 320 soil samples were taken from each 40 sampling points; and 320 plant samples were taken (160 root samples and 160 leaves samples), in 20 sampling points from the cultivated land, in 8 campaigns during April-November period.

The total tetracycline (parent drug +metabolite) amount was determined by ELISA assay method using R-Biopharm Ridascreen® Tetracycline (Art. No. R3501) test kit. This is a competitive enzyme immunoassay for tetracycline, minocycline, rolitetracycline, chlortetracycline and oxytetracycline in meat samples. The method validated for food samples were modified to soil and plant samples. Recoveries were determined by addition of standards for all sample types**.** All reagents required for enzyme immunoassay are contained in the test kit. Sample extraction was carried out with McIllvain buffer, using the method described in the kit with little modification. For sample purification Rida ® C18 column was used. The HPLC grade distillate water was produced by

Millipore Milli-Q academic system. A microtiter plate spectrophotometer (ELISA READER) was used for quantification of absorbance at 450 nm.

RESULTS AND DISCUSSIONS

The results obtained for the TCs screening in the soil and the *P. crispum var. neapolitanum* samples taken in the 8 campaigns (April-October 2012) are presented in table 1.

Table 1

The results obtained for the TCs screening in *P. crispum var. neapolitanum* samples taken in April-October 2012

Value	Soil* µg kg^{-1}		*P. crispum* var. *neapolitanum* samples	
	Location		Root µg kg^{-1}	Leafs µg kg^{-1}
	Cultivated land	Uncultivated field		
Minimum	2.25	0.12	2.35	1.21
Maximum	38.57	4.83	32.06	20.27
Average	21.54	3.22	22.65	15.55

(Fodor et al, 2012).

The obtained data confirmed the fact that TCs accumulate in the soil due to the administration of manure for the agricultural purpose mentioned in the literature (Zheng et al, 2012). The TCs found in the soil in which was added manure is significantly higher than in the uncultivated field (almost seven times).

The vegetables cultivated in those farms can also accumulate TCs. The quantitative accumulation rank is determined by the each species characteristics, stage of development, growing period and plants parts. The present study revealed that in the roots the accumulation rate is higher than in leafs.

CONCLUSIONS

The enzyme immunoassay technique (ELISA) screening methods, makes possible the determination of total active tetracycline molecules. The tetracycline molecular determined as metabolites of furazolidon. This technique can be use for the samples of soil and plant leafs with good results and show attractive features because require just little sample pre-treatment, exhibit high sensitivity, and are inexpensive in comparison to other instrumental analysis.

Tetracycline accumulates in the soil of the agricultural farmlands due to the administration of manure and also in the vegetables cultivated on it in low concentrations µg kg^{-1}. The environmental behaviour and the fate of

TCs in manure, soil and crops after cropland application remain largely unknown (O’Connor, Aga, 2007).

Xenobiotics as TCs, can have impact on living organisms, as any other synthetic chemical we are using in our daily life, and it is present in the environment in measurable concentrations

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