Occurrence of Neonicotinoid Residues in Danube River and Tributaries

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Occurrence and fate of 6 neonicotinoid insecticides belonging to different chemical classes were investigated in the aqueous phase of surface water at 16 sampling locations along the Romanian side of the Danube River and its three main tributaries (Jiu, Olt, Arges). This is the first report on the neonicotinoid occurrence in the Danube River and three tributaries. It was observed a contamination of Danube River and its tributaries, higher in planting period than pre-or post- planting period, with the next compounds (detection frequency and the concentration range): thiamethoxam (68.7%, 0.9-3.8ng/L), clothianidin (64.6%, 0.84-9.6ng/L), nitenpyram (52.08%, 0.39-11.1ng/L), imidacloprid (31.2%, 0.5-8.2ng/L), acetamiprid (16.6%, 0.84-12.7ng/L). The four main neonicotinoids (clothianidin, thiamethoxam, imidacloprid, nitenpyram) follow the classic pattern in which concentrations and frequency increase during the planting period and that is correlated with seed crop treatment. Total neonicotinoid levels present in investigated Rivers, reaching up to 31.6 ng/L, may affect aquatic invertebrates that are most susceptible to these insecticides. For clothianidin and nitenpyram were obtained positive correlations between the percentage of the area planted with cereals and concentrations of this compound (ρ= 0.574, ρ =0.665) which indicate their use in agricultural area. For imidacloprid were obtained positive correlations between percent of permanent cultivated crop in urban land and concentrations of this compound (ρ =0.264, ρ =0.877).

Keywords: neonicotinoids, surface water, SPE-LC-MS/MS, Danube River

Neonicotinoids are extensively used to control pests in a wide variety of crops. They are classified into three families: N-nitroguanidines, nitromethylenes and N-cyanoamidines [1]. Neonicotinoids are a neurotoxic group used in veterinary medicine, urban planning and agricultural lands to protect crops. They can be used by several methods, such as foliar sprays on plants above the soil, or by treating plant roots in the soil or by injecting into tree trunk [2]. They are now used more than any other class of insecticides and comprise approximately one quarter of all insecticides used. They are authorized for application in 120 countries, and imidacloprid accounts for 41% of the market and is the second most applied pesticide in the world. In many countries, neonicotinoids are used as seed-covered crops for crops such as rapeseed, sunflower, grains, beets and potatoes (especially imidacloprid, clothianidin and thiamethoxam). Thiamethoxam and clothianidin are used in agricultural areas and imidacloprid is also used for animal protection and gardening in urban areas. [3]. In 2018 the European Commission approved the use of imidacloprid, thiamethoxam and clothianidin treated seeds only in permanent greenhouse. Thus, the use of these substances in agriculture for agricultural purposes was forbidden [4]. Monitoring results show that neonicotinoids are present in all environmental compartments (water, soil and vegetation). Thus, the persistence and displacement of neonicotinoids into aquatic ecosystems can seriously affect the most sensitive aquatic invertebrates on which vertebrate wildlife depends for food [5]. Neonicotinoids are soluble in water (solubility from 185 to 600,000mg/L, log K_{ow} -0.55 to 1.26), a property that help to function as systemic pesticides which can be taken up by crops. Neonicotinoids are soil persistent, with clothianidin having the longest soil degradation half-life (1386 days, [3, 6]. Neonicotinoids were observed passing into water through direct leaching into ground water and subsequent discharge into surface water during storm events, degradation of treated plant material in water, contact with treated seeds or aerial applications on plants (spray drift on Rivers). Based on aquatic invertebrate species sensitivity, water quality reference values have been established by government agencies in Europe and North America. In the Netherlands and other European countries, the protective level for short-term peak concentrations of imidacloprid is 0.2 µg/L, whereas for long-term exposures the threshold is 8.3 ng/L [7]. The imidacloprid chronic (short- term) invertebrate aquatic life benchmark is 0.23 µg/L in Canada [8] and 13 ng/L in Sweden [9]. The chronic toxicity value at which there is no observable adverse effects is 0.01 µg/L for aquatic insects in U.S.A. [10]. Thus, the chronic exposure benchmark for aquatic insects is 0.01µg/L in conformity with Environmental Protection Agency. The studies on surface water contamination of the Danube River conducted in Romania aimed to detect organic pollutants, metals and inorganic substances resulting from agriculture [11-13]. Therefore, there is a need of data on the presence of these contaminants in the Romanian part of Danube basin and in its tributaries. The purpose of this study is to describe the occurrence of six neonicotinoids (acetamiprid -ACE, clothianidin-CLO, dinotefuran -DIN, imidacloprid -IMI, nitenpyram- NIT and thiamethoxam -THM) along the Romanian part of the Danube River and assess the contribution by its main three tributaries: Jiu, Olt and Arges. The temporal and spatial variation of neonicotinoids in planting period, pre- and post-planting periods was also evaluated. To our knowledge, this study provides the first investigation for monitoring of 6 neonicotinoids in the Danube River in Europe. It is known that the water from the Danube River is the main source of drinking water for the residents along the River. These results are important to assess the potential risk of neonicotinoids on the aquatic and terrestrial organisms.

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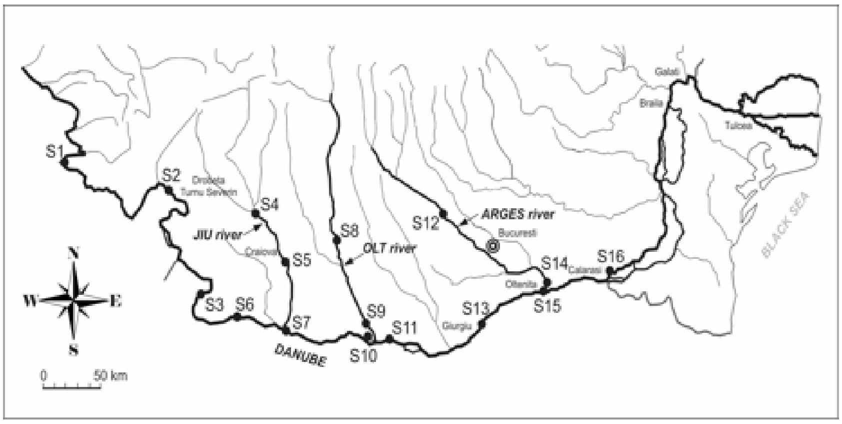

Fig. 1. Locations of sampling points in the Danube River basin

Table 1
SITES NAME AND GPS DATA FOR SAMPLES ON DANUBE RIVER AND TRIBUTARIES

Sampling points	GPS coordinates	Sampling points	GPS coordinates
S1 – Bazias locality on Danube River	44°47'32.61"N 21°23'20.07"E	S9 – Izbiceni, upstream confluence with Danube River	43°48'41.71"N 24°42'27.71"E
S2 – Gura Vaii locality on Danube River	44°40'7.40"N 22°33'10.74"E	S10 – Islaz (Danube), upstream confluence with Olt River	43°42'22.72"N 24°44'4.01"E
S3 – Calafat locality on Danube River	43°57'50.94"N 22°54'15.72"E	S11 – Turnu Magurele (Danube), downstream confluence with Olt River	43°43'2.69"N 24°48'56.65"E
S4 – Filiasi, Jiu River, upstream Craiova	44°34'8.32"N 23°27'18.14"E	S12 – Km 36 upstream Bucharest on Arges River	44°28'45.25"N 25°40'47.87"E
S5 – Podari, Jiu River downstream Craiova	44°15'18.48"N 23°47'25.08"E	S13 – Giurgiu (Danube), upstream confluence with Arges River	43°52'37.50"N 25°58'49.92"E
S6 – Rast (Danube), upstream confluence with Jiu River	43°51'24.84"N 23°17'18.79"E	S14 – Chirnogi on Arges River	44° 6'38.09"N 26°38'15.33"E
S7 – Bechet (Danube), downstream confluence with Jiu River	43°45'11.32"N 23°56'30.69"E	S15 – Oltenita, downstream confluence with Arges River	44° 3'51.89"N 26°38'45.49"E
S8 – Olt River downstream Slatina / bridge	44°23'29.63" N 24°21'4.84"	S16 – Calarasi (Danube)	44° 8'15.69"N 27°20'8.26"E

Experimental part

Sample collection

The samples were collected in the periods 5-7 February, 16-18 April, 29-31 May 2018 from 10 points of the Danube River and from 2 points from some tributaries, as shown in figure 1. Each time, surface water samples were collected from a depth of 0.5 m and at a distance of 2 m from the bank of the River. River water samples were taken in 500 ml bottles of brown glass with a screw cap. After collection, the samples were transported at 4°C to laboratory and processed in 48 hours. Sites name and GPS coordinates are presented in table 1. At the beginning of April, crops of corn, sunflower, soybeans are planted with treated seeds, and in May during crop growth other neonicotinoids may be applied directly to plants against pests.

Sample treatment and analysis of the neonicotinoids

Analytical procedure was based on our previously reported method [14]. Briefly, water treatment involves solid phase extraction (SPE) with an enrichment factor of 500 according to the previously published method. Water samples (500 mL) were filtered on 0.45 µm glass fiber filters. After that, the filter was washed with methanol in order to pass all analytes in filtrate. The water samples were pre-treated with 280 Dionex-Autotrace automated solid-phase extraction (SPE) apparatus (Thermo-Scientific). The SPE was consisted of: cartridge conditioning with methanol and ultrapure water; water samples loading on the cartridge at a flow-rate of 10 mL/min. To eliminate potential interference, the cartridge sorbent was washed with ultrapure water. After that, the sorbents were dried with air and then the contaminants were eluted with methanol. Then, the methanol extract was evaporated to dryness using an Turbo-Vap LV (Caliper Life Sciences Inc., Hopkinton, MA, USA) with a nitrogen gas. The obtained residue was reconstituted with 0.5mL acetonitrile: formic acid 0.2% (90/10, v/v). Chromatographic determinations were done with an Agilent 1260 series LC system (Waldbronn, Germany). Detection of the analytes was performed with an 6410B mass spectrometer (triple quadrupole, Agilent Technologies, Waldbronn, Germany) equipped with electrospray ionization source (ESI). The separation of neonicotinoids was performed on a Hypersil-Gold column (150 x 2.1 mm, 3.5 µm, Thermo-Scientific), at 20°C, using a gradient of mobile phase (acetonitrile: 0.2% formic acid).

Results and discussions

Neonicotinoid occurrence

A total of 48 of surface water samples (30 on Danube River, and 6 on each of the rivers: Arges, Jiu, Olt) influenced by agriculture and potential by WWTP (waste water treatment plant) effluents were analysed, using the SPE-LC-MS/MS method for neonicotinoid insecticides detection. The lowest concentration obtained corresponded to nitenpyram (0.39 ng/L, in Danube River, Islaz point) and the highest to acetamiprid (12.7ng/L, in Arges River, Chirnogi point). From contaminants investigated in surface water samples, the next compounds were frequently detected in multiple samples at low concentrations with decreasing frequency thiamethoxam (68.7%), clothianidin 64.6%), nitenpyram 52.08%, imidacloprid (31.2%). In contrast, the much less used acetamiprid and dinotefuran were detected with lowest frequency of (16.6% and 2% respectively, table 2). Although the frequency of determination is probably correlated to the usage pattern on farms and residential areas, it is also related with the persistence of the individual compounds in water, especially with their hydrolysis half-life. Under acidic and neutral pH conditions compounds are stable, only under high alkaline conditions (*p*H 9 or above, not expected in surface water) hydrolysis can occur [6]. Also, imidacloprid, clothianidin and other neonicotinoids are frequently found in waters because they are resistant to hydrolysis. Clothianidin (Soil DT50 1386), thiamethoxam (Soil DT50 72), imidacloprid (soil DT50 228), dinotefuran (Soil DT50 100) are persistent in soil with higher reported DT50 values. We observed that neonicotinoid concentrations detected in surface water samples in spring 2018 were probably due to spring rains that transport neonicotinoids from the cultivated soil with treated seeds, mainly by runoff from the fields. The highest concentrations were recorded in the Arges River, Chirnogi Point (12.7ng/L acetamiprid) followed by nitenpyram 11.1ng/L in Jiu River, Podari point.

Figure 2a-2d shows the variation of individual neonicotinoid concentration versus location along Danube River and in Jiu, Olt and Arges tributaries. In figure 2e are shown the total neonicotinoid concentration in every sampling point in April month.

Table 2
RANGE AND MEDIAN OF NEONICOTINOIDS CONCENTRATIONS AND DETECTION FREQUENCY OF INDIVIDUAL COMPOUNDS IN SURFACE WATER SAMPLES

Compound	Range (ng/L)	Median	Detection frequency (%)
Thiamethoxan	0.9-3.8	1.6	68.7
Clothianidin	0.84-9.6	2.5	64.6
Nitenpyram	0.39-11.1	1.3	52.08
Imidacloprid	0.5-8.2	2.9	31.2
Acetamiprid	0.84-12.7	1.3	16.6

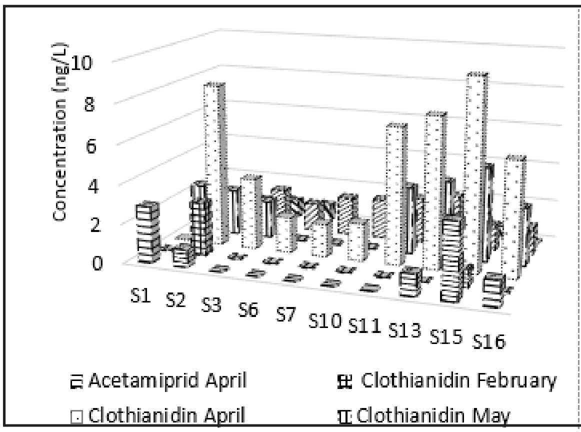

Fig. 2a. Acetamiprid, clothianidin, nitenpyram, concentrations in Danube River

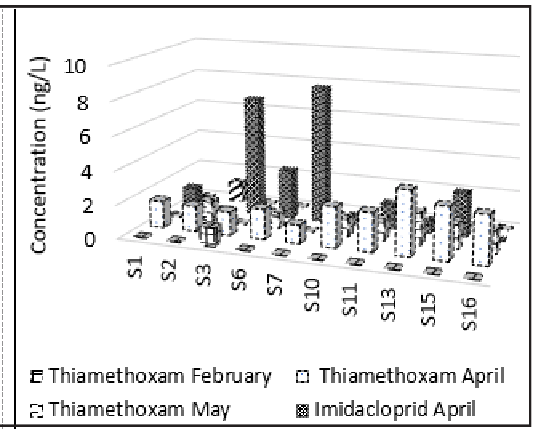

Fig. 2b. Thiamethoxam, imidacloprid concentrations in Danube River

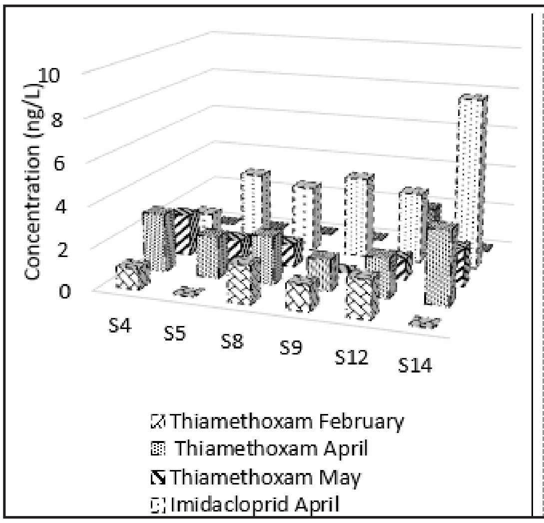

Fig. 2c. Thiamethoxam, imidacloprid, dinotefuran concentrations in Arges, Jiu, Olt Rivers

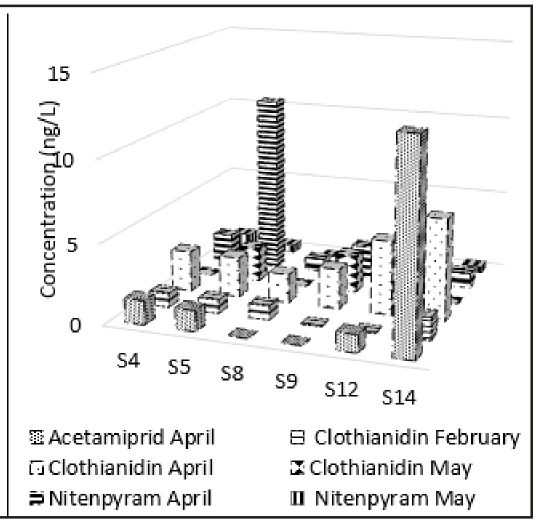

Fig. 2d. Acetamiprid, clothianidin, nitenpyram concentrations in Arges, Jiu, Olt Rivers

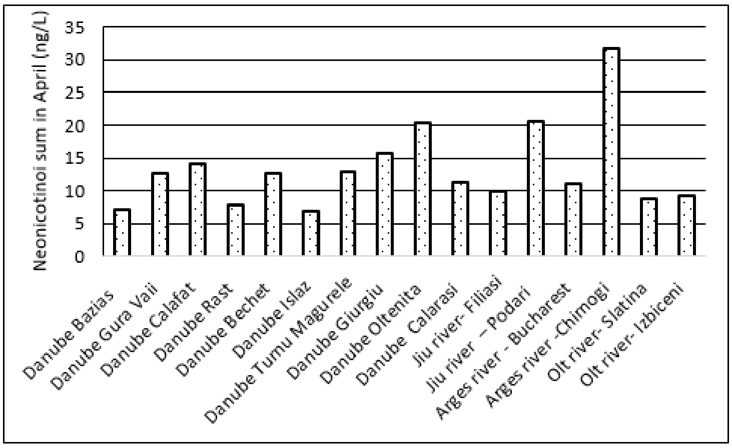

Fig. 2e. Neonicotinoid concentrations sum in each sampling point from Danube River and tributaries in planting period

Individual discussion on Danube River and tributaries

During the monitoring period, it was observed that Danube River was contaminated with clothianidin (0.5ng/ L -8.1ng/L), thiamethoxan (0.9ng/L-3.8ng/L), nitenpyram (0.39 -2.6 ng/L, acetamiprid (0.89-3.8 ng/L), imidacloprid (0.5-8.1ng/L). The highest frequency of neonicotinoids detection in Danube River was observed in April month, 33.3% for nitenpyram and thiamethoxam, followed by 30% for clothianidin and 26.6% for imidacloprid. The highest increase of neonicotinoids concentrations between sampling periods was observed for imidacloprid (27-fold higher in April versus February) and clothianidin (10.6-fold higher in April versus February). Rapid water dissipation was observed for acetamiprid whom concentrations decreased by 6-fold in May comparative to April. These compounds appear to be transported through River because concentrations of them were found in the most sampling points in during the same period [3]. Co-presence of clothianidin and thiamethoxam with similar values may be due to their use in the same basins but also to the fact that clothianidin is the degradation product of thiamethoxam. In the case of the Arges River, it was observed pollution with acetamiprid (1.1-12.7ng/L), thiamethoxam (0.98-3.5ng/L), clothianidin (1.4-6.3 ng/L), imidacloprid (3.4-8.2ng/L), nitenpyram (0.39-0.93ng/L). Dinotefuran was detected in one sample only from Arges River in February at 1.5ng/L, this compound having the lowest frequency detection of 2%. The Arges River was the most contaminated from all investigated Rivers having a neonicotinoid sum of 31.6ng/L in Chirnogi point, in planting period. Acetamiprid value increased 21-fold in planting period versus February month. Imidacloprid increased 27-fold in April in comparation with February. The highest values were 12.7ng/L acetamiprid followed by 8.2ng/L imidacloprid. Acetamiprid half-life is 34 days for water photolysis and 2-20 days in soil [6]. Probably due to these properties acetamiprid was not detected in the other two monitoring months. Thiamethoxam which is considered to have moderate persistence (up to 72 days soil half- life and 11.5 days hydrolysis half-life at alkaline pH) was detected in all periods in Arges River. Jiu River showed contamination with clothianidin (0.84-2.55ng/L), thiamethoxam (1 -2.8ng/L), nitenpyram 0.5-11.1 ng/L, imidacloprid (1.3-3.6ng/L), acetamiprid 1.3-1.5ng/L. The neonicotinoid sum in planting month (April) in downstream was (20.5ng/L in Jiu River, Podari location) twice highest than in upstream (9.9ng/L in Filiasi location). It can be supposed that there can be a possible contribution of WWTP in neonicotinoids for this value, higher in downstream than upstream [15]. After Arges River, Jiu River was the second as most contaminated, with neonicotinoid, as can see in figure 2 together with Danube River in Oltenita location which had sum value of 20.4ng/L in April. The highest increase of neonicotinoids concentrations in planting month (April) than in pre-planting (February) was observed for nitenpyram, whose concentration increased 37-times. Nitenpyram values were up to 22 times higher in April (11ng/L Jiu River, Podari point) than in May (0.5ng/L in Jiu River, Podari point). In Olt River were detected imidacloprid (3.2-3.9ng/L), thiamethoxam (1.1-2.3 ng/L), nitenpyram (0.78-2 ng/L), clothianidin (0.86-2.5ng L). The sum of neonicotinoids concentrations was similar in Olt River in the two sampling locations from April (8.7 and 9.2ng/L). The imidacloprid concentration increased 13-fold in April compared to February. The persistence of clothianidin (0.86ng/L) both during the pre-planting and in the growing period (2.4ng/L) can be attributed to its long half-life in the soil 545 days. The spring rains carry this compound through leaching and runoff of agricultural terrains into surface water and in ground water. Hydrolysis is not expected to occur in surface water with neutral *p*H (hydrolysis occurs only at alkaline *p*H) and photo-degradation takes place only in clear waters in their surface layer; rainfall increases the turbidity of water Rivers and does not allow photodegradation [16].The neonicotinoid concentrations in surface water are a cause of concern, because it suggests that soil values, where these substances are used, are accumulating over a period of time (years) [17]. The imidacloprid and thiamethoxam insecticides have been observed to be mobile in soil with a high possibility to flow through the soil profile laterally through the soil flow path to pollute surface water [2]. The neonicotinoids persistence in Rivers is in function of the exposure to sunlight the soil or water *p*H and temperature. The fact that neonicotinoids remain in surface waters all-year-round suggests that biodegradation, while possible, is very limited and probably not occurring in most environments. Imidacloprid and thiamethoxam degraded rapidly only in high alkaline water (pH 9 or above), while in neutral environment (*p*H 7) they remained stable. The frequent determination of thiamethoxam, clothianidin, imidacloprid can be due to some factors such as: intensive use, long soil degradation half-life (50-1386 days).

The levels of neonicotinoids detected in Danube River and tributaries are lower than environmental concentrations observed in other countries. Thus, neo-nicotinoid concentrations and frequency in the River of Osaka city (Japan), were higher than we reported in our study for acetamiprid (1.4 ng/L, 9.5%), clothianidin (7.8-100 ng/L, 83%), dinotefuran (31-100 ng/L, 100%), imidacloprid (7-25ng/L, 66%) and thiamethoxam (3.2-11 ng/L, 75%) [18]. In the USA, streams from corn and soybean areas the values determined during growing period were much higher than our study (frequency, maximum, median): clothianidin (75%, 257ng/L, 8.2n/L) imidacloprid

(23%, 42ng/L, <2ng/L), thiamethoxam (47%, 185ng/L, <2ng/L) [3]. The maximum imidacloprid concentrations (0.5-82 ng/L) detected in our study were much lower than values reported for Surface water in Netherlands where imidacloprid had maximum concentrations of 320µg/L [19]. The clothianidin (0.84-9.6 ng/L) concentrations were similar with values reported in a Hungarian study for surface water (17-40 ng/L) [20]. The concentrations and frequencies observed for imidacloprid in our study (0.5-8.2ng/L; 31%) were situated in the same range with the values reported in Guadalquivir River Basin (2.3-19.2 ng/L, 17-58%, Spain) [21]. Also, in Sweden, imidacloprid was detected in 36% of the sampling points, this value being similar to the detection rate of 31.2% obtained in our study [9].

Temporal variation

In order to obtain a better understanding of the neonicotinoids temporal variation, the collection was divided into three periods based on time crop planting: pre-planting (February), planting (April), growing season (May). This division shown temporal variation of neonicotinoids levels and frequency. The four neonicotinoids (clothianidin, thiamethoxam, imidacloprid, nitenpyram) respects the pattern in which concentrations and frequency's increase in April correlated with crop planting period. Analyzing the temporal variation in detail it was observed low neonicotinoids frequency detection (acetamiprid, clothianidin, thiamethoxam, imidacloprid) in Rivers during pre-planting period, prior to their application in April. Thus, the total neonicotinoids level was 16.3 ng/L in February much less than in April (212 ng/L). Detections in River prior to new application in April can be due to uses during the previous growing season (fig. 3). Comparing the levels of neonicotinoids in the three sampling months it observes a clearly increase of their concentrations. With the onset of cultivation, the neonicotinoids were frequently detected in the majority of sampling points. Thus, their levels increase at maximum values of 12.7 ng/L acetamiprid, nitenpyram 11.1 ng/L, clothianidin 9.6 ng/L, imidacloprid 8.2 ng/L. Analyzing the averages of the monthly concentrations is noticed that neonicotinoids values obtained in April month (which is the period for planting of cereals and vegetables), were clear higher than in the February month (pre-planting period). Thus, thiamethoxam was detected with average concentrations of 2.34 ng/L, in April and 0.64 ng/L in May; in February it wasn't detected in any samples. The acetamiprid average concentration was 2.96 ng/L only in April. Clothianidin showed average concentrations of 4.49 ng/L in April, 1.26 ng/L in February and 2.85 ng/L in May. Imidacloprid had higher average concentration in planting month (3.4 ng/L) than in growing month (1.2 ng/L). At the time of transition from the planting period to the growth period, the frequency of detection neonicotinoids and their levels decreased considerably (fig. 3). Thus, total neonicotinoid concentration decreased from 211 ng/L in April to 52 ng/L in May. This seems to be due to the period of time between planting the treated seeds and growing the plant. Thus, the neonicotinoids level detected in growing period is due to runoff from soil seed treated (after storms events) and to possible foliar applications.

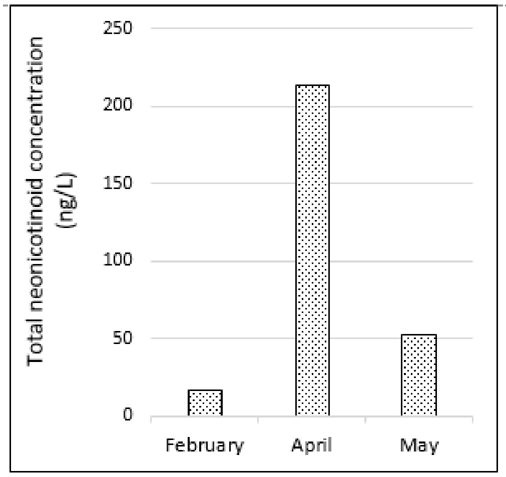

Fig. 3. Total neonicotinoid concentrations versus sampling month

Table 3

WATERSHED CHARACTERISTICS INCLUDING LAND USE ASSOCIATED WITH SAMPLING LOCATION FOR NEONICOTINOID INSECTICIDES ON DANUBE RIVER

Sampling points	Land use (%)			Detection frequency (%)				Maximum concentration (ng/L)			
	Agriculture (corn, wheat, oat)	Urban (residential gardens)	Permanent crops (fruit, vineyards)	CLO	NIT	THM	IMI	CLO	NIT	THM	IMI
Danube R. - Bazias	59.7	35.4	4.8	33.3	66.6	33.3	33.3	2.4	1.49	1.6	1.2
Danube R. - Gura Vaii	41	26.5	32.8	100	66.6	33.3	33.3	8.25	1.95	1.5	1.2
Danube R. - Calafat	35.2	34.7	29.8	66.6	66.6	66.6	33.3	3.6	2	1.4	7.1
Danube R. - Rast	37.6	31.5	26.9	33.3	66.6	66.6	33.3	1.8	1.4	1.8	2.9
Danube R. - Bechet	41.3	29.5	27.5	33.3	33.3	33.3	33.3	1.6	1.9	1.1	8.1
Danube R. - Islaz	39.6	31	29.3	33.3	66.6	66.6	33.3	1.9	2	2.4	0.5
Danube R. - Turnu Magurele	43.8	33.1	26.1	66.6	66.6	66.6	33.3	3.4	2.3	2.3	1.5
Danube R. - Giurgiu	42.7	37.3	19.9	66.6	33.3	66.6	33.3	7.6	2.6	1.9	0.6
Danube R. - Oltenita	53.2	18.04	28.4	100	66.6	66.6	33.3	9.6	1.3	3.1	2.6
Danube R. - Calarasi	55.9	16.2	26.3	66.6	33.3	66.6	-	5.8	1.4	2.9	-

Table 4
SPEARMAN'S RANK CORRELATIONS BETWEEN NEONICOTINOIDS CONCENTRATIONS AND LAND USE FOR DANUBE WATERSHED

Compound	Agriculture (corn, wheat, oat)	Urban (residential gardens)	Permanent crops (fruit trees, vineyards)
Thiamethoxan	-0.702	-0.222	-0.639
Clothianidin	0.574	-0.560	0.230
Imidacloprid	-0.452	0.264	0.877
Nitenpyram	-0.250	0.665	0.020

Neonicotinoid correlations with land use

Clothianidin, thiamethoxam and nitenpyram were detected with high frequency and concentrations in agricultural land (table 3). The maximum concentrations of clothianidin (9.25ng/L in Oltenita point and 8.25ng/L in Gura Vaii point), nittenpyram (2.6ng/L in Giurgiu point, 2.3ng/L in Turnu Magurele point) thiamethoxam (3.1ng/L in Oltenita point, 2.9ng/L in Calarasi point) were obtained in locations with over 35% -56% of the land cultivated with grains [22]. Thus, for clothianidin were obtained positive correlations between percent of cultivated grains and concentrations (ρ = 0.574, table 4) which indicate their use in agricultural area. For imidacloprid were obtained positive correlations percent of cultivated crop in urban land and concentrations (ρ=0.264) similar with other studies that reported higher concentrations of these neonicotinoid in urban area [3]. Also, a strong correlation was obtained for imidacloprid between percent of permanent crops and concentration (ρ = 0.877) which indicates its use for protection of fruit trees and vineyards. In the case of nitenpyram it was observed a positive correlation between percent of cultivated urban land and concentration (ρ=0.665) which suggest its use in this area. For thiamethoxam was calculated a negative correlation between concentrations and percent of grain cultivated (ρ = -0.702). These relationships were similar to results reported for clothianidin and imidacloprid in a US study [17].

By calculating the correlation coefficient (ρ =0.219) between clothianidin and thiamethoxam concentrations in the same sampling points, a positive value was obtained, which indicate that the two neonicotinoids are used together in the same areas.

Conclusions

It was observed that neonicotinoids are persistent and mobile in environment this fact being due to their agricultural use and precipitations which transport compounds from soil to rivers. Neonicotinoid were more frequent detected in higher concentrations in planting period than in other seasons. The most contaminated surface waters were Arges followed by Jiu and Danube River. Temporal variation shows a pulse of neonicotinoid in correlation with crop planting period. Compounds were frequently detected in multiple samples at low ng/L concentrations with decreasing frequency as follow: thiamethoxam, clothianidin, nitenpyram, imidacloprid, acetamiprid, dinotefuran. Although the concentrations of imidacloprid were below the acceptable level of chronic toxicity in the European Union (8.3 ng/L), however, the total neonicotinoid levels present in investigated Rivers reaching up to 31.6 ng/L one sample may affect aquatic invertebrates that are most susceptible to these insecticides. These neonicotinoids may still pose a risk to aquatic organisms and the insectivorous population and action should therefore be taken to prevent these problems.

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