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Original article

Occurrence of dicarboximidic fungicides and their metabolites' residues in commercial compost

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Abstract – In this work we analysed samples of different commercial composts to verify the presence of metabolites of dicarboximidic fungicides (Iprodione, Procymidone, Vinclozolin and Chlozolinate) widely used in agriculture as botritycides. The commercial compost extracts, obtained by sonication with acetonitrile, were analysed by HPLC-DAD-MS. The detection limits (DL) of the analytical method were appraised for all considered compounds and for both the detectors. The analysis confirmed the presence of fungicide residues (i.e. Iprodione, 48 μg/kg) in three samples of commercial compost and traces of some corresponding metabolites (i.e. Metabolite I of Procymidone, 20 μg/kg) for most examined matrices. These results underline a possible presence of these fungicides and corresponding metabolites in soil, after the addition of compost.

dicarboximidic fungicides / metabolites / commercial compost / HPLC-DAD-MS

Résumé – Fungicides dicarboximidiques dans le compost commercial. Ce travail comporte l'analyse des métabolites de fongicides dicarboximidiques utilisés comme botritycides (Iprodione, Procymidone, Vinclozolin, Chlozolinate) dans différents échantillons de compost commerciaux. Les extraits des composts ont été analysés par HPLC-DAD-MS (chromatographie en phase liquide – détecteur à série de diodes – spectromètre de masse). Les analyses ont confirmé la présence de résidus de fongicides dans trois composts commerciaux (ex. 48 μg/kg d'iprodione) et des traces de certains métabolites dans la plupart des échantillons (ex : 20 μg/kg du métabolite I du Procymidone). Ces résultats suggèrent que l'application de compost favorise la présence des fongicides et/or des métabolites spécifiques dans le sol traité.

fongicides dicarboximidiques / métabolites / compost commerciaux / HPLC-DAD-MS

1. INTRODUCTION

The use of fungicides is vital for effective control of plant diseases that are estimated to cause yield reductions of almost 20% in major worldwide food and cash crops [5]. Generally vegetables undergo several treatments with these substances during their growth, but their correct application does not produce a residual content in the food products higher than that consented by legal limits [2–4, 9]. Nevertheless, consistent residues of fungicides and their metabolite products may be found in crops and environmental matrices as a consequence of incorrect or too-frequent use [1, 4]. This becomes very important when refuse, caused by anthropogenic activities, such as vegetable waste, urban sludge and agro-industrial waste, is destined for the production of compost that is employed in the soil to maintain or to increase its fertility. During the composting process, the green matrix is subjected to an aerobic biodegradation, during which pesticides (if present) may also undergo a transformation in relative metabolites. This possibility has been verified in our previous investigations [12, 13] for Iprodione, Procymidone, Vinclozolin and Chlozolinate, four dicarboximidic pesticides widely used in agriculture as botritycides. Their chemical structures and those of corresponding breakdown products are summarised in Table I.

Moreover, compost is a matrix rich in organic substances that may interfere in pesticides' determination. Even after a selective extraction step, the compost extract is still very rich in organic compounds that absorb in the UV-Vis range as well as analytes, thus causing low resolutions and noise increase. This fact makes a qualitative evaluation of these compounds very difficult and their quantification with the UV-vis detectors impossible. The use of Mass Spectrometry (a more selective and versatile detection method) has been proposed to possibly overcome the problem and to be able to quantify the real presence of dicarboximidic fungicides and their corresponding metabolites in a matrix as complex as compost.

Thus, the aim of this work was the development of an analytical method, based on the use of HPLC-MS, suitable for identifying and quantifying the presence of residues of some dicarboximidic fungicides together with their degradation products in different commercial compost samples, because of

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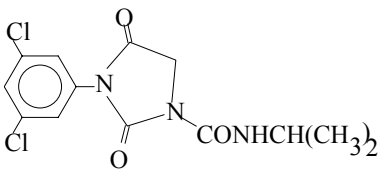
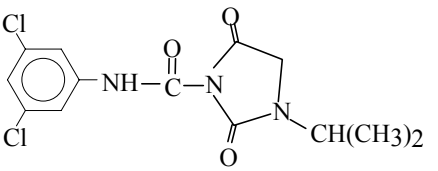
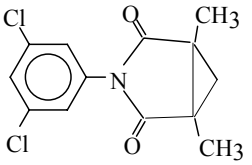
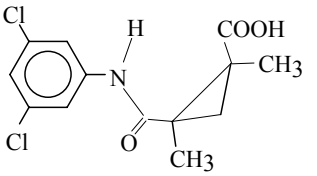
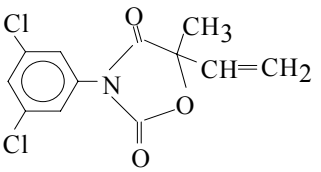
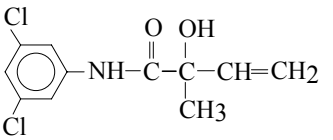
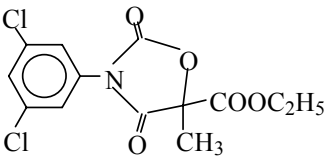
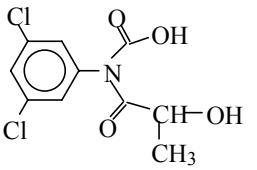
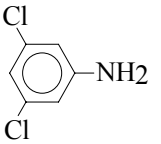
Table I. Chemical formulas of some dicarboximidic fungicides and relative metabolites.

FUNGICIDE	PM_n	Formula	Metabolite	PM_n	Formula
IPRODIONE	329		Isomer	329	
PROCYMIDONE	283		Metabolite I	301	
VINCLOZOLIN	285		Metabolite V_1	259	
CHLOZOLINATE	331		Metabolite C_2	277	
			Common Metabolite: 3,5-DCA	161	

the widespread use of compost and the danger of a possible bioaccumulation of active ingredients and relative metabolites in the soil treated with it.

2. MATERIALS AND METHODS

2.1. Chemicals

Iprodione [3 - [(3,5 - dichlorophenyl)–N - (1 - methylethyl) - 2,4–dioxo–1 - imidazolidinecarboxyamide] (purity 98%) and Vinclozolin [3 - (3,5 - dichlorophenyl)–5–methyl–5–ethenyl - 2,4 - oxazolidinedione] (purity 98.4%) were from Supelco (USA). Procymidone [N - (3,5 - dichlorophenyl) - 1,2 - dimethylcyclopropane 1,2 - dicarboximide], (purity 99.7%), Chlozolinate [ethyl–3 - (3,5 - dichlorophenyl)–5–methyl - 2,4–dioxo - 1,3–oxazolidine–5 - carboxylate] (purity 98.5%) and 3,5 – dichloroaniline (3,5-DCA) (purity 97%) were supplied by Dr Ehrenstorfer GmbH (Germany).

The preparation of the other metabolites (Metabolite I, Isomer, Metabolite V_1 and Metabolite C_2), not commercially available, was conducted by an abiotic process working in controlled conditions, reported elsewhere [12,13]. The purity and the concentration of the relative reference solutions were verified through the HPLC-DAD-MS system.

The solvents used were Acetonitrile supergradient-grade (G. Chromasolv, Riedel de Haën) and ultra-pure water, obtained from water purification Elgastat systems.

2.2. Procedure

The commercial compost samples (5 kg) were kindly supplied by A.R.P.A. (Regione Piemonte-Assessorato Ambiente) and were sampled according to the coning and quartering method [8]. It was necessary to grind and to subject the gross sample to a further screening with 4-mesh sieves in order to increase the homogeneity of three subsamples (100 g) and to withstand the attack by the solvent (acetonitrile) used to extract analytes. Then they were preserved at –18 °C in polyethylene bags.

In Table II the product composition of analysed commercial composts is reported. Among these, the compost indicated as No. 0 in Table II was chosen as the reference matrix for its maturation time, which is greater than one year, and after having verified that the analytes' quantity is below the detection limit (DL) of our detection method.

Table II. Product composition of analysed commercial compost samples.

Sample (No.)	Product composition
0	Reference compost: poplar barks
1	Barks+ pruning residues+urban and industrial sludges
2	Barks+ pruning residues+urban and industrial sludges
3	Barks+ pruning residues+urban and industrial sludges
4	Barks+ pruning residues+urban and industrial sludges
5	Barks+ pruning residues+selected organic wastes
6	Barks+ pruning residues+selected organic wastes
7	Vegetable residues

Samples were extracted with acetonitrile by using the ultrasound technique, according to a method previously optimised [14]. This procedure shows a good efficiency of extraction for all the considered analytes (80–95%), with intervals of uncertainty, expressed as the Relative Standard Deviation (RSD%), of less than 7% for all examined matrices.

For the chromatographic separation and identification of analytes the HPLC-DAD-MS system was used, constituting a liquid chromatograph (HP Series 1100, LC), equipped with a 1100 Binary Pump, connected in series to a UV-Vis/Diode Array Detector (HP Series 1100, DAD) and to a Mass Spectrometer (HP Series 1100, MS) with a single quadrupole. The ionisation sources used were Atmospheric Pressure Chemical Ionization (APCI) and Electrospray (ESI). A Lichrosphere (HP, Agilent) RP-C18 column (250 × 2 mm, 5 μm, 1 i.d.) at room temperature was used for the analysis at a flow rate of 0.3 ml/min. Gradient elution was employed with acetonitrile/water previously filtered and degassed. The solvent programming was as follows: initially 1 min isocratic with 40% acetonitrile, 34 min linear gradient to 70% acetonitrile, then 5 min isocratic with 70% acetonitrile. The absorption of analytes was monitored at 210 nm and the entire absorbance data was recorded from 190 to 400 nm, during every analysis.

The analytical procedure consisted of initial HPLC separation of the extracts, which were previously filtered (0.2 μm, with PTFE filters), followed by the evaluation of the purity of the chromatographic peak through the plot of isoabsorbance of DAD and finally, the identification and the quantification of the analytes through the MS detector, by using the APCI or ESI as ionisation sources. The quantification of peaks was carried out by an external standard method, which included using measurements of peak areas and a calibration curve for each pesticide.

The limit of detection (DL) was defined as the concentration corresponding to the blank signal plus three times the standard deviation of the same blank (level of confidence of 99.87%) [6, 7]. For both the detectors, data reproducibility was expressed as RSD%, with at least three probes.

3. RESULTS AND DISCUSSION

The high complexity of the matrices made the optimisation of operative conditions necessary for the identification of analytes in MS, through Flow Injection Analysis (FIA) – opportunely varying for both the sources, polarity and other parameters according to the structure of the considered molecule. This step is fundamental before the evaluation of the detection limits (DL). The optimisation occurred whilst working with standard solutions and in scan mode, with mass range 50–250 m/z.

In Table III the optimised operative parameters are reported.

For the determination of active ingredients and some metabolites (with low or medium polarity) it was sufficient to vary the polarity of the APCI source, while for the other metabolites, characterised by high polarity, the influence of the structure made the use of the ESI source necessary. These results show that there is a variety of conditions to detect fungicides with MS, even if their structures are different only for some radicals. For corresponding metabolites not only are operative conditions very different, but so too is the ionisation source. In fact, for fungicides the ionisation source is always the APCI, while for breakdown products there is also the ESI.

Table III. Operative conditions optimised for analyte detection with MS.

Fungicide metabolite	Procymidone	Metabolite I	Iprodione	Isomer	Chlozolinate	Metabolite C_2	Vinclozolin	Metabolite V_1	3,5-DCA
Source	APCI	ESI	APCI	APCI	APCI	ESI	APCI	ESI	APCI
Polarity	Positive	Negative	Positive	Positive	Negative	Negative	Negative	Negative	Positive
Drying gas flow (L/min)	7.0	10.0	7.0	7.0	5.0	10.0	5.0	10.0	7.0
Neb. Press. (psig)	60	60	60	60	60	60	60	60	60
Drying gas T (°C)	300	350	300	300	350	350	350	350	300
Vcap (V)	3000	3000	4000	4000	4000	4000	4000	3000	3000
Vfram.(V)	90	70	50	50	80	50	60	70	90
Corona (μA)	10	/	10	10	55	/	55	/	10
Vap. T (°C)	325	/	325	325	325	/	325	/	325
Monitoring ions	284/286	300/302	330/332	330/332	286/288	276/278	241/243	258/260	162/164

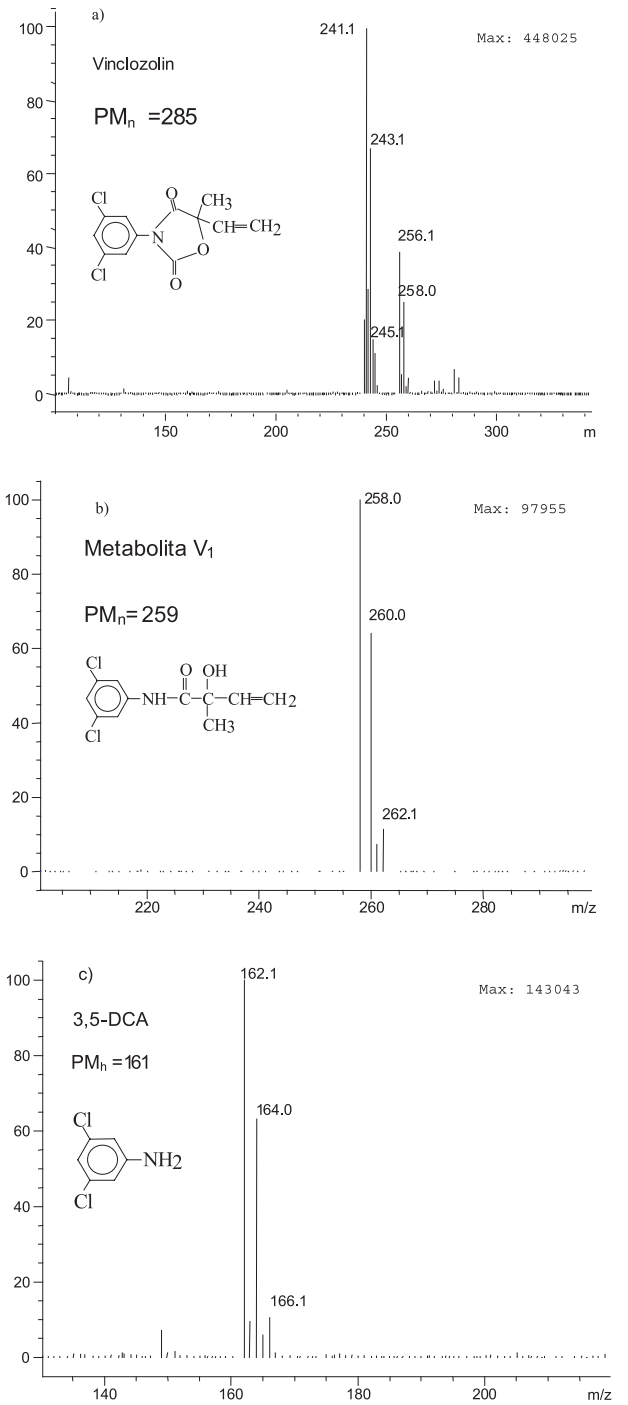


Figure 1. Mass spectra of (a) Vinclozolin, (b) its metabolite V_1 and (c) common metabolite 3,5-dicloroaniline (DCA).

These data underline that, to evaluate the presence of fungicides and relative metabolites in commercial compost samples, it is still not possible to work in the same operative conditions with a MS detector. This fact, on the one hand, allows us to detect each analyte in an optimal way, but on the other, slows down the analysis time.

In Figure 1, three mass spectra of Vinclozolin (a) and of its specific metabolite V_1(b) and 3,5-dicloroaniline (DCA) (c) are reported.

As can be noted, for Vinclozolin in APCI working in negative polarity, it was not possible to get the formation of a molecular ion, but a basic peak is present (Fig. 1a), very probably due to the loss of a propane molecule. For the corresponding metabolites (V_1 and 3,5-DCA), however, it was possible to obtain the respective molecular ions with the relative triplet by only working with different ionisation sources and polarities, respectively, ESI in negative polarity and APCI in positive polarity, as reported in Figures 1 (b) and 1 (c).

The optimisation step of operative conditions for analyte detection in MS allowed us to achieve DL values lower than those obtained with a DAD detector, as shown in Table IV. In this table the concentrations of the instrumental DL (obtained through straight line calibration by using standard solutions of the analytes) and the concentrations of the analytical method DL (obtained by standard additions to the extract of compost No. 0) are reported.

It is worth noting an increase in the Procymidone and Iprodione analytical DL for the DAD detector in the presence of composting material, even forty-fold compared with those obtained for the instrumental DL.

The values of the analytical DL of the compost No. 0 (both for MS and DAD) were then compared with those obtained with the other composts. The DL for compost No. 1 is reported in Table IV as an example, because all considered compost samples showed a similar DL, within the experimental error.

Obviously, there is an increase in DL values both for the DAD detector and for the MS detector for all analytes, as reported in Table IV, with only one exception, represented by the metabolite V_1 in MS. These results confirm that commercial compost is not only a very complex matrix, but also a material that can make the determination of dicarboximidic fungicides complicated as well as their metabolites, because of its variable composition, by causing a variable noise profile. In Figure 2, three chromatograms for Procymidone in a standard solution and in two different compost extracts are compared. A progressive increase in the DAD noise can be noted, due to the increase in the complexity of the matrix, which makes the determination of the fungicide in the extract of compost No. 1 more difficult, as reported in Figure 2 (c).

In Figure 3 the Total Ion Current (TIC) profiles are reported. The presence of Procymidone and many peaks at different retention times (t_r) can be observed. These peaks correspond to substances present in compost, that ionised forming charged chemical types with a m/z ratio equal to that of the fungicide, by causing an increase in analysis difficulty. Thus, every compost sample must be considered as a new matrix, able to interfere more or less in the determination of pesticides and relative breakdown products.

Since the DL values are much lower for the MS detector, the presence (or absence) of analytes was confirmed in the last analysis from the MS. The analyte quantification occurred after construction of calibration curves with a range of concentration included between 1 μg/kg and 50 μg/kg, with good correlation,

Table IV. Instrumental DL and analytical method DL evaluated for analytes with DAD and MS detectors.

FUNGICIDE	Instrumental DL (µg/kg ± RSD%)		Analytical method DL Blank (compost No. 0) (µg/kg ± RSD%)		Analytical method DL Matrix effect (compost No. 1) (µg/kg ± RSD%)	
Metabolite	DAD	MS	DAD	MS	DAD	MS
PROCYMIDONE	5 ± 2.9	100 ± 3.1	200 ± 7.4	200 ± 6.5	>500	500 ± 4.8
Metabolite I	20 ± 2.4	5 ± 2.9	300 ± 6.1	5 ± 5.5	500 ± 7.1	500 ± 6.1
IPRODIONE	5 ± 2.7	5 ± 2.5	100 ± 4.2	20 ± 4.6	500 ± 4.9	500 ± 4.2
Isomer	5 ± 2.3	5 ± 3.3	50 ± 4.1	5 ± 5.2	>500	10 ± 5.1
VINCLOZOLIN	5 ± 2.9	50 ± 2.4	50 ± 3.9	50 ± 4.5	>500	100 ± 6.2
Metabolite V_1	10 ± 2.4	1 ± 2.7	100 ± 5.1	1 ± 5.0	>500	1 ± 6.9
CHLOZOLINATE	5 ± 3.1	5 ± 5.2	50 ± 6.5	5 ± 5.2	100 ± 6.3	50 ± 7.2
Metabolite C_2	10 ± 4.	10 ± 3.0	> 500	50 ± 6.9	>500	500 ± 7.3
3,5-DCA	5 ± 2.1	20 ± 3.7	50 ± 4.7	50 ± 3.9	500 ± 9.4	500 ± 6.4

expressed as r^2 ± RSD% (0.99987 ± 7.2%). As shown by the results of the tested compost reported in Table V, MS analysis allowed us to detect not only very low quantities of some fungicides; for example, Iprodione in compost No. 2 and No. 3, but also traces of some metabolites – for example, the metabolite V_1 in compost No. 2 (2.3 ppb) and in compost No. 5 (2.7 ppb). In the same Table V, it is also shown that some pesticides and metabolites were present, even if below their relative DL. Vinclozolin and Chlozolinate were detected in compost No. 6 and compost No. 7, respectively. Furthermore, we observed the presence of a metabolite in a sample contaminated by the relative pesticide (Iprodione isomer in compost No. 4), but also the presence of Metabolite I in compost No. 5, where the relative pesticides were not detected.

These results are in accordance with those obtained for these fungicides by other researchers in different matrices, such as food products [2–4, 9].

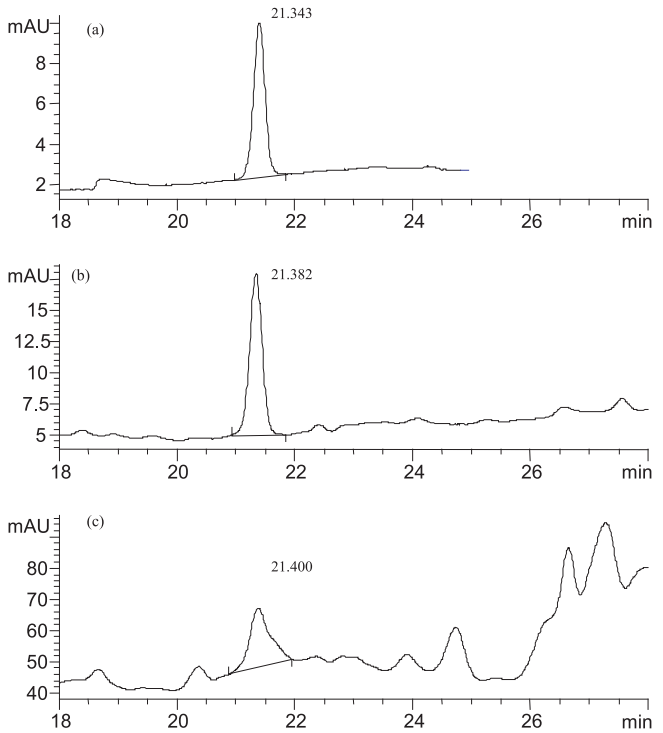


Figure 2. Comparison of Procymidone chromatograms: (a) 200 µg/kg standard; (b) extract from compost No. 0 spiked with 200 µg/kg; (c) extract from compost No. 1 spiked with 200 µg/kg.

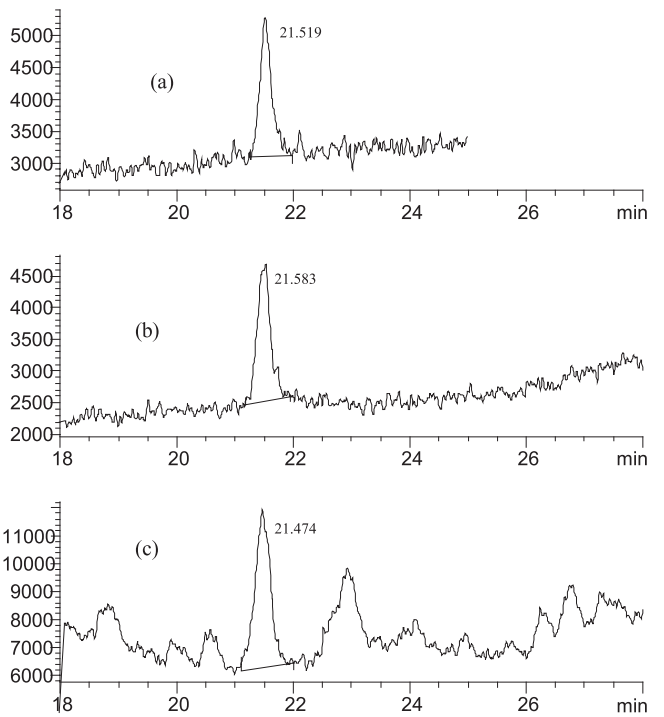


Figure 3. Comparison of Procymidone TIC: (a) 200 µg/kg standard; (b) extract from compost No. 0 spiked with 200 µg/kg; (c) extract from compost No. 1 spiked with 200 µg/kg.

Table V. Quantity of analytes found in commercial compost samples. The bar indicates not detectable signals, whereas <DL means results inferior to the analytical DL.

FUNGICIDE Metabolite	Compost No. 1 (μg/kg ± RSD%)	Compost No. 2 (μg/kg ± RSD%)	Compost No. 3 (μg/kg ± RSD%)	Compost No. 4 (μg/kg ± RSD%)	Compost No. 5 (μg/kg ± RSD%)	Compost No. 6 (μg/kg ± RSD%)	Compost No. 7 (μg/kg ± RSD%)
PROCYMIDONE	/	/	/	/	/	/	/
Metabolite I	/	20 ± 5.4	/	/	<DL	/	/
IPRODIONE	/	48 ± 7.3	21 ± 7.8	/	/	/	/
Isomer	/	/	<DL	/	/	/	/
VINCLOZOLIN	/	/	/	/	/	<DL	/
Metabolite V_1	/	2.3 ± 6.1	/	/	2.7 ± 6.8	/	/
CHLOZOLINATE	/	/	/	/	/	/	<DL
Metabolite C_2	/	/	/	/	/	/	/
3,5-DCA	/	/	/	/	/	/	/

4. CONCLUSIONS

The use of the two detectors DAD and MS consecutively made it possible to verify the effective contamination of some commercial compost samples and to proceed with a correct quantitative evaluation of the presence of both the residues and the metabolites of the fungicides examined. The alternate use of APCI and ESI sources for mass spectrometry reduced the problem due to the high complexity of the matrices. By using SIM modality, it was possible to confirm or exclude the presence of the analytes in different extracts and to reduce the matrix interference that was in any case very evident with DAD. This is also confirmed by the values obtained by the detection limit (DL) in MS (Tab. V).

The concentrations of the dicarboximidic fungicides in the analysed commercial composts came out lower than the values normally indicated in the law pertinent to the application of pesticides on different crops [10]. In accordance with the law, the limits go from a minimum value for Iprodione equal to 0.05 mg/kg (ppm) to a maximum value for Chlozolinate of 10 mg/kg (ppm). By comparing the quantities found in compost samples with the Lethal Dose values (L.D. 50) reported in the bibliography [11], it is clear that they do not reach particularly toxic levels (they are about 10 000 times less). On the contrary, for almost all of the metabolites, data relative to the limits of tolerance and real toxicity were not found in the literature, thus it has been impossible to make any comments about the danger they pose. Currently, the only available value is concerning 3,5-Dicloroaniline (DCA) (L.D. 50 = 1600 mg/kg mouse). This compound is defined as toxic if inhaled, ingested or absorbed through the skin, and can also be declared dangerous for its cumulative effects.

The results obtained show that composting material acts as a probable source of pollutants for the soil. Therefore, because the compost is employed as a fertiliser for the soil in a concentration of 1% according to full field applications and 25% for those in floriculture, the possibility of their bioaccumulation in treated soils must not be ignored.

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REFERENCES

[1] Barbero L., Gaia P., Enological consequence of the use of Ronilan in grape cultivation, Vini Ital. 21 (1979) 95–100.

[2] Cabras P., Diana P., Meloni M., Pirisi F.M., Residue determination of some insecticides and fungicides on grapes by reversed-phase high-performance liquid chromatography, J. Agric. Food Chem. 30 (1982) 569–572.

[3] Flori P., Stanzani R., Musacci P., Zironi R., Atti Giorn. Fitopatol. 2 (1982) 13–22.

[4] Flori P., Zironi R., Brandolini V., Musacci P., in: Clementi F. (Ed.), Atti convegno Fitofarmaci e ambiente Cagliari, 7–8 October 1982, Paretti, Florence, pp. 89–98.

[5] Gullino M.L., Leroux P., Smith C.M., Uses and challenges of novel compounds for plant disease control, Crop Prot. 19 (2000) 1–11.

[6] Kateman J., Pijpers F.W., Quality Control in Analytical Chemistry, in Elving P.J., Winefordner J.D. (Eds.), New York, (1981), pp. 72–78.

[7] Massart D.L., Vandeginste B.G.M., Deming S.N., Michotte Y., Kaufman L., Chemometrics: a textbook, Elsevier, Amsterdam (1998).

[8] Woodget B.W., Cooper D., Samples and Standards, ACOL Editors: N.B. Chapman, John Wiley and Sons, London (1991).

[9] Molinari G.P., Stringari G., Flori P., Atti Giorn. Fitopatol. (Suppl. 1) (1980) 97–100.

[10] Normativa Nazionale, Ordinanza Ministeriale, Ministero della Sanità, S.O.G.U. No. 202, August (1990).

[11] The Pesticide Manual (1990).

[12] Vanni A., Gamberini R., Calabria A., Determination and identification of metabolites of the fungicides Vinclozolin and Chlozolinate in compost, Toxicol. Environ. Chem. 78 (2000) 11–30.

[13] Vanni A., Gamberini R., Calabria A., Nappi P., Determination and identification of metabolites of the fungicides Iprodione and Procymidone in compost, Chemosphere 41 (2000) 1431–1439.

[14] Vanni A., Gamberini R., Calabria A., Pellegrino V., Determination of presence of fungicides by their common metabolite, 3,5-DCA in compost, Chemosphere 41 (2000) 453–458.