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Assessment of Pesticide Content in Apples and Selected Citrus Fruits Subjected to Simple Culinary Processing

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Abstract: Over the span of the last decade, certain pesticides have been banned in apple tree and citrus tree cultivations. Hence, it is important to conduct research focused on estimating the occurrence of residues of pesticides from the perspective of compliance with the relevant legislative regulations. Equally important is to estimate the reduction in pesticide residues through simple procedures such as washing and peeling. This research was conducted in the years 2012 and 2020. An assessment was made of the effect of in-house processing, such as conventional washing with tap water and peeling, on the level of pesticide residues in apples and citrus fruits (oranges, grapefruits and lemons). The level of pesticide residue was determined with the use of the QuEChERS method of extraction in conjunction with LC-MS/MS analysis. One can clearly observe a smaller number of pesticides identified in the edible parts of fruits in 2020 (seven pesticides in apples and three in citrus fruits) compared to 2012 (26 pesticides in apples and 4 in citrus fruits). In apples from 2012, only in the case of disulfoton was the maximum residue limit (MRL) exceeded, while in samples of apples from 2020 no instance of exceeded MRL was noted. This study did not reveal exceeded MRL values in the edible parts of citrus fruits in the analysed years. The absence of detected instances of pesticides not approved for use in the analysed years indicates that the producers complied with the relevant legislative regulations. The results obtained indicate that conventional washing with water (about 1.5 L/one fruit) did not have any effect on the level of pesticide residues in the analysed fruits. Apple peeling allowed for a reduction in pesticide levels in the range of 24% (carbendazim) to 100% (triflumuron, thiodicarb, tebuconazole).

Keywords: pesticide residues; fruits; fruit peel; culinary processing; washing

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1. Introduction

Apples and citrus fruits are extensively consumed in many countries, and they are considered to be valuable health-promoting food as they contain biologically active components such as ascorbic acid, carotene (provitamin A) and group B vitamins, flavonoids, carotenoids, and phenolic acids, which have beneficial effects for human health [1–4]. Systematic consumption of fruits and vegetables reduces the risk of civilisation diseases and also facilitates body mass control [5–8]. Reports of the World Health Organisation of the Food and Agriculture Organisation recommend that adults should consume at least five portions of fruits and vegetables daily [9,10]. On the other hand, cultivations of apple trees and citrus trees are attacked by numerous pathogenic fungi and by pests. For this reason, plant protection agents are commonly applied in conventional cultivations (as well as in sustainable agriculture systems), at various stages of plant development, to control pests and diseases which can cause a reduction in yields [11]. Residues of pesticides in fruits originating from such cultivations may constitute a hazard for human health [12–14].

European Union (EU) regulations specify the maximum residue limit (MRL) for pesticides in food of plant origin [15]. In the United States (US), the US Environmental Protection Agency (EPA) approves and registers the use of pesticides and establishes the maximum level of pesticide chemical residues (tolerances) allowed to remain in or on food after treatment with approved pesticides (tolerances may be known as MRL in other countries) [16]. It should be mentioned that the values of MRL are updated on the basis of current data on the safety of application of such plant protection agents, and within the last decade, certain pesticides have been banned from use in crop plant cultivations. Incorrect use of pesticides may result in food contamination, and in consequence, cause harm to consumers, therefore it is important to monitor the level of residues of those contaminants in fruits. Knowledge of the effect of home processing on the level of pesticide residues in fruits is necessary to reduce dietary hazards.

Numerous studies have demonstrated the presence of pesticide residues in apples and citrus fruits, and although home processing of fruits, such as cooking, frying, baking and blanching, leads to a considerable reduction in pesticide residues [17], those fruits are most often consumed with no prior processing. Apples are often eaten directly after washing, or they are peeled, but it needs to be emphasised that certain consumers eat apples even without washing them first. For apple washing under tap water to be effective, a sufficient amount of water must be used, and the duration of the washing process is also important. In model experiments, apple washing time of 2 minutes is specified [18]. However, it should be emphasised that consumers often do not wash fruits at all, or else they wash them for a notably shorter time, e.g., 5–10 s, which results from various reasons, such as the high speed of life or ecological considerations. In the case of apples, the peel can be easily removed by peeling, which will also remove most of the pesticide residues, however, important nutrients (e.g., polyphenolic compounds, fibres, pigments, vitamins and minerals) will be lost as well [18]. In the case of citrus fruits, it is routine procedure to peel them before eating, with the exception of lemons, which are often used unpeeled for spice purposes. The aim of the study was to conduct an estimation of pesticide residues in various parts of apples and citrus fruits, taking into account different aspects such as the effect of simple culinary procedures, the type of washing under tap water and peeling, and keeping in mind the compliance of fruit producers with changes in the relevant legislative regulations.

2. Materials and Methods

2.1. Experimental Material

The test material consisted of fresh fruits: apples (cultivars Jonagold, Gala, Gloster, Rubin, Jonagored, Szampion, Ligol, Alwa, Golden Delicious), oranges, grapefruits (red) and lemons, purchased from markets in Lublin, Eastern Poland (22°34′ E, 51°15′ N). The apples were from a Polish production, while the citrus fruits were imported (Spain, Cyprus, Turkey, South Africa). The fruit samples originated from the harvests of 2012 (nine batches of apples and two batches each of oranges, grapefruits and lemons, respectively) and 2020 (five batches of apples and two batches each of oranges, grapefruits and lemons, respectively), which allowed for observations, important for the experiment, concerning the analysis of pesticide residues in fruits over a long time period.

2.2. Culinary Processing

Prior to the analyses, the fruits were subjected to simple culinary processing routinely performed in households:

- Apples: the test material consisted of whole fruits, unpeeled and unwashed, apple peel and flesh, and also samples of whole apples after simple washing in a stream of cold tap water for 15 s (about 1.5 L/1 fruit, the time of washing under tap water was shorter than in the model experiment [18], which was aimed at reproducing actual conditions of apple washing in households).

- Citrus fruits: the conventional procedure for preparation for consumption was applied, i.e., the test samples consisted of peeled fruits, in this case samples of peel and flesh, without prior washing of the fruits.

2.3. Chemicals

High-purity pesticide standards (250) were used for testing (98–99%, Dr. Ehrenstorfer GmbH, Augsburg, Niemcy; ChemService, West Chester, PA, USA): 2,4,5-T, 2,4-D, 2,4-DB, 3,5-dichloroaniline, 3-hydroxycarbofuran, abamectin, acephate, acetamiprid, acrinathrin, alachlor, aldicarb, aldicarb sulfoxide, aldicarb sulphone, ametryn, amitraz, atrazine, azinophos-ethyl, azinophos-methyl, azoxystrobin, benfuracarb, bentazon, benzoylprop ethyl, bifenazate, bromacil, bromoxynil, bromuconazole, buprofezine, butoxycarboxin, cAp (captan), carbaryl, carbendazim, carbetamide, carbofuran, carbosulfan, carboxin, chlorantraniliprole, chloridazon, chlorotoluron, chlorpyrifos, chlorsulfuron, clofentezine, clomazone, clothianidin, coumaphos, cyanazine, cyanofenphos, cycloate, cymoxanil, cyphenothrin, cyprofuram, dEf (decafentin), demeton-S-methyl, demeton-S-methylsulphon, desethyl atrazin, desisopropyl atrazin, desmedipham, desmetryn, diafenthiuron, dialifos, diazinon, dicamba, dichlofluanid, dichloprop (2.4-dP), dichlorvos, dicrotophos, diflubenzuron, dimefuron, dimethachlor, dimethenamide, dimethoate, dimethomorph, diniconazole, diphenamide, diphenylamine, disulfoton, ditalimfos, diuron, dMf (2,4-dimethyl-phenyl-formamidine), dodine, epoxiconazole, etaconazole, ethiofencarb, ethirimol, ethofenprox, etoxazole, etrimphos, fenamidon, fenamiphos, fenazaquin, fenbuconazole, fenhexamid, fenoxap-p-ethyl, fenoxycarb, fenpropimorph, fenpyroximate, fenthion, fenthion sulfon, fenuron, fipronil, flazasulfuron, florosulam, fluazifop, fluazifop-p-butyl, fluazinam, fludioxonil, flufenacet, flufenoxuron, fluometuron, fluroxypyr, flurtamon, fluthiacet methyl, flutriafol, fonofos, fosthiazate, fuberidazol, furathiocarb, halfenprox, haloxyfop, haloxyfop methyl, haloxyfop-2-ethoxyethyl, heptenophos, hexaflumuron, hexazinone, hexythiazox, imazalil, imazamox, imazapyr, imidacloprid, indoxacarb, ioxynil, iprodione, iprovalicarb, isazofos, isocarbamide, isomethiozin, isoproturon, isoxaflutole, lenacil, linuron, lufenuron, malaoxon, malathion, mCpA (2-methyl-4-chlorophenoxyacetic acid), mCpB (4-(2-methyl-4-chlorophenoxy) butyric acid), mCpP (mecoprop), mecarbam, mepanipyrim, metalaxyl, metalaxyl-M, metamitron, metazachlor, metconazol, methabenzthiazuron, methacrifos, methamidophos, methidathion, methiocarb, methoprotryne, methoxyfenozide, metobromuron, metolachlor, metolachlor S, metosulam, metoxuron, metrafenon, monocrotophos, monolinuron, monuron, myclobutanil, nicosulfuron, nitenpyram, norflurazon, novaluron, omethoate, oxamyl, oxycarboxin, oxydemethon methyl, paraoxon ethyl, paraoxon methyl, parathion ethyl, pebulat, penconazole, pencycuron, phenkapton, phenmedipham, phenothrin, phenthoate, phorate, phosalone, phosmet, phosphamidon, phoxim, picoxystrobin, pirimicarb, pirimiphos methyl, prochloraz, profenofos, prometryn, propamocarb, propanil, propaquizafop, prophos, prosulfuron, pyraclostrobin, pyraflufen ethyl, pyridaphenthion, pyridate, pyrimiphos ethyl, pyriproxyfen, quinmerac, quizalofop-p-ethyl, resmethrine, rimsulfuron, sebuthylazin, sethoxydim, siltiopham, simazine, simetryn, spinosad A, spinosad D, spirotetramat, spiroxamin, sulfotep, sulprofos, tebuconazole, tebufenozide, tebufenpyrad, tebutam, teflubenzuron, tepraloxydim, terbucarb, terbumeton, terbuthialzine desethyl, terbuthylazine, tetramethrin, thiabendazole, thiacloprid, thiamethoxam, thiodicarb, thiophanate methyl, tolclofos methyl, tolylfluanid, triadimefon, tri-allate, triamiphos, triazophos, trichlorofon, triclopyr, trifloxystrobin, triflumuron, triforine. Standard solutions of pesticide in acetonitrile, with a concentration of approximately 1000 mg/L, were prepared. Next, standard solutions of a mixture of pesticides in acetonitrile, with concentration of about 35 mg/L, were prepared for each of the compounds. Working standard solutions were prepared by diluting the standard mixtures of pesticide solutions with acetonitrile. All standard solutions were stored at temperatures lower than −20 °C. The choice of analysed pesticides resulted from the demand of apple and citrus producers' customers for analyses in line with the laboratory services market in the region. In addition, only pesticides for which the criteria for analytical quality were met were included in the analysis.

2.4. Preparation of Samples, Analytical Methods and Instrumentation

The analytical procedure was described in detail in earlier works [19,20]. The content of pesticide residues in the analysed samples (in 2012 and 2020) was assayed following a modified procedure developed in accordance with the standard PN-EN 15662:2008 [21], with the use of the QuEChERS method combined with LC-MS/MS analysis, a Shimadzu Prominence/20 series HPLC system (Shimadzu, Tokyo, Japan) and AB SCIEX 4000 QTRAP® LC-MS/MS system with Turbo V source (Foster City, CA, USA). Both transitions were used for quantification and confirmation purposes (see the Supplementary Material: Tables S1 and S2). The procedure applied in the study has been approved by the Polish Centre of Accreditation (PCA AB 1375).

In addition, in 2020, the tested samples of apples and citrus fruits were analysed at the AGROLAB laboratory (Deblin, Poland) for the content of the following pesticides: boscalid, chlorpyrifos–methyl with the use of the GC-MS/MS method, pyrimethanile, spirotetramat-monohydroxy, sum spirotetramat-enol, -ketohydroxy, -monohydroxy, -enol-glucosid, THPI (tetrahydrophthalimide), flonicamid, TFNG (N-(4-trifluoromethylnicotinoyl)glycine) and TFNA (4-(trifluoromethyl)nicotinic acid) with the use of the LC-MS/MS method, in conformance with the methodology described in the standard PN-EN 15662:2018-06. The procedure applied in the study has been approved by the Polish Centre of Accreditation (PCA AB 444).

2.5. Statistical Analysis

Data were analysed using one way ANOVA followed by Duncan's test using the SAS statistical system (SAS Version 9.1, SAS Inst., Cary, NC, USA). The significance of all tests was set at $p \leq 0.05$.

3. Results and Discussion

3.1. Pesticide Residues in Apples and Citrus Fruits—Comparison

3.1.1. Apples

Figures 1 and 2 present the content of pesticide residues in apples from the harvests of 2012 and 2020, respectively. Table 1 presents data concerning the levels of the individual pesticides in the analysed apples, taking into account the values of MRL and the limit of quantification (LOQ).

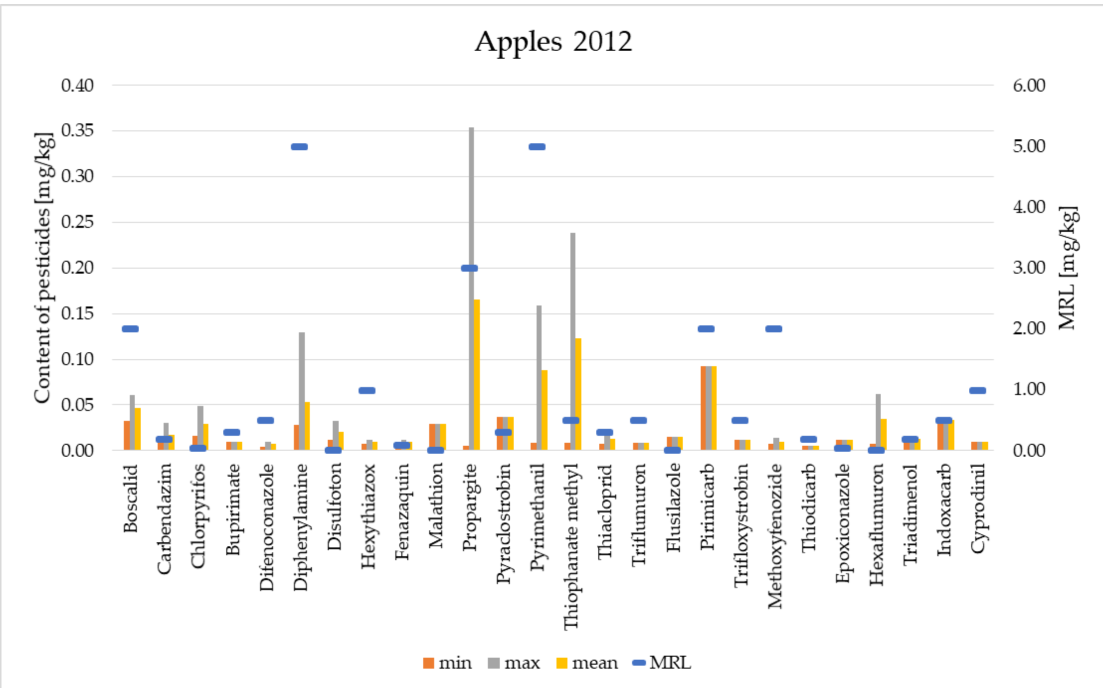

Figure 1. Content of pesticides in samples of whole apples from the harvest of 2012.

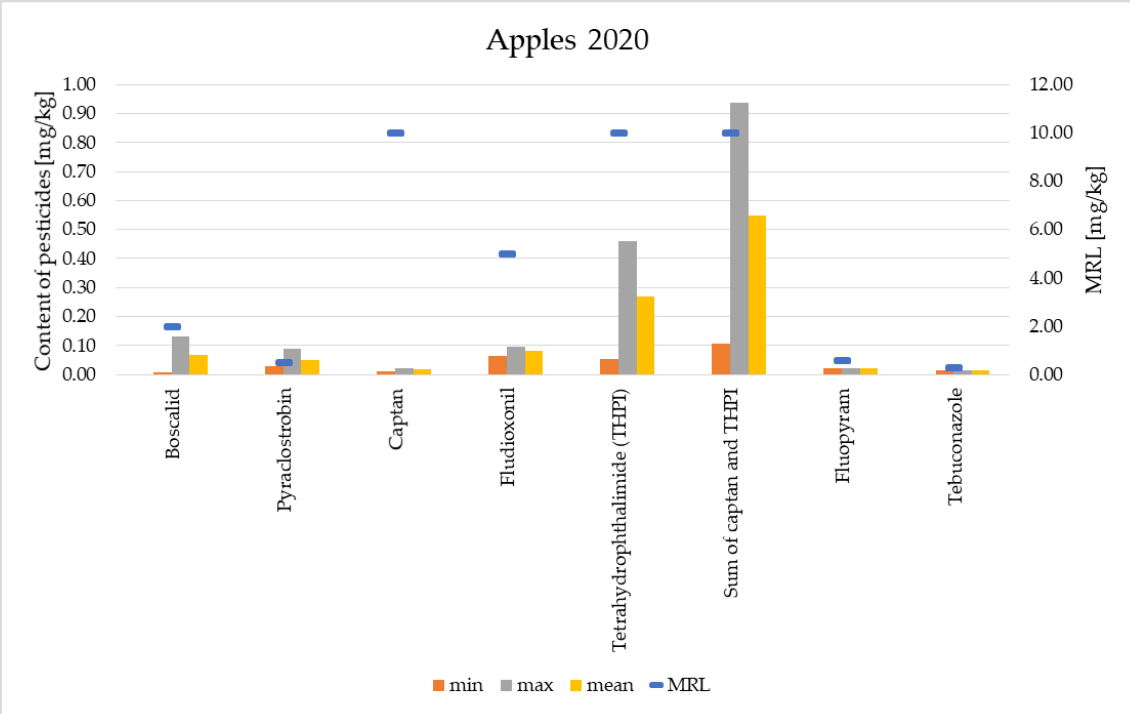

Figure 2. Content of pesticides in samples of whole apples from the harvest of 2020.

In the samples of whole apples from 2012, the number of identified pesticides was 26: boscalid (fungicide), carbendazim (fungicide), chlorpyrifos (acaricide, insecticide), bupirimate (fungicide), difenoconazole (fungicide), diphenylamine (plant growth regulator), disulfoton (insecticide), hexythiazox (acaricide, insecticide), fenazaquin (acaricide), malathion (acaricide, insecticide), propargite (acaricide), pyraclostrobin (fungicide, plant growth regulator), pyrimethanil (fungicide), thiophanate methyl (fungicide), thiacloprid (insecticide), triflumuron (insecticide), flusilazole (fungicide), pirimicarb (insecticide), trifloxystrobin (fungicide), methoxyfenozide (insecticide), thiodicarb (insecticide), epoxiconazole (fungicide), hexaflumuron (insecticide), triadimenol (fungicide), indoxacarb (insecticide), and cyprodinil (fungicide), as shown in Figure 1, Table 1. In contrast, only seven pesticides were identified in whole apples from the 2020 harvest: boscalid (fungicide), pyraclostrobin (fungicide, plant growth regulator), captan (fungicide), fludioxonil (fungicide), tetrahydrophthalimide (THPI) (metabolite of captan–fungicyd), fluopyram (fungicide), tebuconazole (fungicide), as shown in Figure 2, Table 1. One can clearly note that the number of pesticides identified in apples in 2020 was lower. Only two pesticides, boscalid and pyraclostrobin, were identified in both analysed batches of whole apples from 2012 and 2020.

The group of pesticides most often identified in 2012 was that of insecticides (50%), followed by fungicides (46%) and growth regulators (8%). In 2020, only fungicides were identified in whole apple samples. This may be due to the fact that at that time there was an increased interest in alternative methods of plant protection, including fruit production in a sustainable system, where one of the primary assumptions is conducting activities aimed at the creation of biological equilibrium. Numerous studies indicate that side effects of chemical control include the destruction of natural flora and fauna and the contamination of water, soil and air, which has an impact on plant resistance to diseases and pests [11,60,61].

Table 1. Levels of individual pesticides in apples and the values of MRL.

Pesticide	Group	Range of Pesticides Concentration				MRL		LOQ mg/kg
		Whole Apples		Apple Peels				
		2012	2020	2012	2020	2012	2020	
boscalid	F	<LOQ–0.0613 d	<LOQ–0.1300 c	<LOQ–0.3245 b	<LOQ–0.7820 a	2 mg/kg [22]	2 mg/kg [23]	0.0005
carbendazim	F	<LOQ–0.0298 b	<LOQ c	<LOQ–0.1691 a	<LOQ c	0.2 mg/kg [24]	not approved	0.0001
chlorpyrifos	A, I	<LOQ–0.0490 b	<LOQ c	<LOQ–0.2685 a	<LOQ c	0.05 mg/kg [25]	not approved 0.01 mg/kg [26]	0.0001
bupirimate	F	<LOQ–0.0098 b	<LOQ c	<LOQ–0.5000 a	<LOQ c	0.3 mg/kg	0.3 mg/kg [27]	0.0001
difenoconazole	F	<LOQ–0.0096 b	<LOQ c	<LOQ–0.0432 a	<LOQ c	0.5 mg/kg [28]	0.8 mg/kg [29]	0.0002
diphenylamine	PGR	0.02820–0.130 b	<LOQ c	0.1536–0.6773 a	<LOQ c	5 mg/kg [30]	not approved 0.05 mg/kg [31]	0.025
disulfoton	I	<LOQ–0.0321 b	<LOQ c	<LOQ–0.1858 a	<LOQ c	0.01 mg/kg [32]	not approved	0.0001
hexythiazox	A, I	<LOQ–0.0119 b	<LOQ c	<LOQ–0.068 a	<LOQ c	1 mg/kg [33]	1 mg/kg [33]	0.0001
fenazaquin	A	<LOQ–0.0117 b	<LOQ c	<LOQ–0.044 a	<LOQ c	0.1 mg/kg [34]	0.1 mg/kg [35]	0.0005
malathion	A, I	<LOQ–0.0291 b	<LOQ c	<LOQ–0.1534 a	<LOQ c	0.02 mg/kg [36]	0.02 mg/kg [37]	0.0001
propargite	A	<LOQ–0.3540 b	<LOQ c	<LOQ–1.4943 a	<LOQ c	3 mg/kg [30]	not approved 0.01 mg/kg [38]	0.0001
pyraclostrobin	F, PGR	<LOQ–0.0370 d	<LOQ–0.087 c	<LOQ–0.1928 b	<LOQ–0.4470 a	0.3 mg/kg [39]	0.5 mg/kg [40]	0.0002
pyrimethanil	F	<LOQ–0.1590 b	<LOQ c	<LOQ–0.6658 a	<LOQ c	5 mg/kg [39]	15 mg/kg [38]	0.0005
thiophanate methyl	F	<LOQ–0.2380 b	<LOQ c	<LOQ–1.3310 a	<LOQ c	0.5 mg/kg [24]	not approved	0.0005
thiacloprid	I	<LOQ–0.0161 b	<LOQ c	<LOQ–0.0867 a	<LOQ c	0.3 mg/kg [41]	not approved 0.3 mg/kg [35]	0.0001
triflumuron	I	<LOQ–0.0082 b	<LOQ c	<LOQ–0.0508 a	<LOQ c	0.5 mg/kg [25]	not approved 0.5 mg/kg [42]	0.0001
flusilazole	F	<LOQ–0.0145 a	<LOQ b	<LOQ–0.0132 a	<LOQ b	0.02 mg/kg [22]	not approved 0.01 mg/kg [43]	0.0002
pirimicarb	I	<LOQ–0.092 b	<LOQ c	<LOQ–0.5001 a	<LOQ c	2 mg/kg [44]	0.5 mg/kg [45]	0.0001
trifloxystrobin	F	<LOQ–0.0116 b	<LOQ c	<LOQ–0.0793 a	<LOQ c	0.5 mg/kg [41]	0.7 mg/kg [46]	0.0001

Table 1. *Cont.*

Pesticide	Group	Range of Pesticides Concentration				MRL		LOQ mg/kg
		Whole Apples		Apple Peels				
		2012	2020	2012	2020	2012	2020	
methoxyfenozide	I	<LOQ–0.0136 b	<LOQ c	<LOQ–0.0932 a	<LOQ–0.0190 b	2 mg/kg [47]	2 mg/kg [48]	0.0001
thiodicarb	I	<LOQ–0.0056 b	<LOQ c	<LOQ–0.0346 a	<LOQ c	0.2 mg/kg [30]	not approved 0.01 mg/kg [49]	0.0001
epoxiconazole	F	<LOQ–0.0116 b	<LOQ c	<LOQ–0.075 a	<LOQ c	0.05 mg/kg [39]	not approved	0.0001
hexaflumuron	I	<LOQ–0.0615 b	<LOQ c	<LOQ–0.321 a	<LOQ c	0.01 mg/kg [15]	not approved	0.0001
triadimenol	F	<LOQ–0.0128 b	<LOQ c	<LOQ–0.0791 a	<LOQ c	0.2 mg/kg [22]	not approved 0.2 mg/kg [50]	0.001
indoxacarb	I	<LOQ–0.0338 b	<LOQ c	<LOQ–0.2153 a	<LOQ c	0.5 mg/kg [51]	0.5 mg/kg [52]	0.0002
cyprodinil	F	<LOQ–0.0094 b	<LOQ c	<LOQ–0.0588 a	<LOQ c	1 mg/kg [33]	2 mg/kg [53]	0.001
fenpyroximate	A	<LOQ b	<LOQ b	<LOQ–0.0332 a	<LOQ b	0.3 mg/kg [28]	0.3 mg/kg [54]	0.0001
iprovalicarb	F	<LOQ b	<LOQ b	<LOQ–0.0269 a	<LOQ b	0.05 mg/kg [30]	0.01 mg/kg [55]	0.0001
lufenuron	I	<LOQ b	<LOQ b	<LOQ–0.0282 a	<LOQ b	0.5 mg/kg [30]	not approved 1 mg/kg [54]	0.0002
bentazon	H	<LOQ b	<LOQ b	<LOQ–0.0311 a	<LOQ b	0.1 mg/kg [36]	0.03 mg/kg [40]	0.001
teflubenzuron	I	<LOQ b	<LOQ b	<LOQ–0.0286 a	<LOQ b	1 mg/kg [25]	not approved 1 mg/kg [40]	0.0001
captan	F	-	<LOQ–0.0230 b	-	<LOQ–0.396 a	3 mg/kg [33]	10 mg/kg (sum of captan and THPI, expressed as captan) [56]	0.01
fludioxonil	F	-	<LOQ–0.0970 b	-	<LOQ–0.5300 a	5 mg/kg [28]	5 mg/kg [40]	0.0001
tetrahydrophthalimide (THPI)–captan metabolite	F	-	0.0530–0.4600 b	-	0.3360–2.6610 a		10 mg/kg (sum of captan and THPI, expressed as captan) [56]	0.01
fluopyram	F	-	<LOQ–0.0220 b	-	<LOQ–0.1310 a	0.6 mg/kg [36]	0.6 mg/kg [46]	0.01
tebuconazole	F	-	<LOQ–0.0130 b	-	<LOQ–0.081 a	1 mg/kg [57]	0.3 mg/kg [58]	0.0001
acetamiprid	I	-	<LOQ b	-	<LOQ–0.128 a	0.7 mg/kg [39]	0.4 mg/kg [59]	0.0001

F—fungicide, H—herbicide, I—insecticide, A—acaricide, PGR—plant growth regulator, LOQ—limit of quantification. a, b, c, . . . —values designated with the same letters in line do not significantly differ at 5% error (Duncan's test).

In the analysed samples of apples from 2012, cases of disulfoton levels higher than the MRL (0.01 mg/kg) [32] were only noted in four samples (0.0204 mg/kg, 0.0268 mg/kg, 0.0314 mg/kg and 0.0321 mg/kg); in four other samples of apples, the levels corresponded to the value of MRL or were only slightly higher (0.0113 mg/kg, 0.0114 mg/kg, 0.0125 mg/kg and 0.0154 mg/kg). Earlier legislation, i.e., the Commission Regulation (EC) No. 149/2008 of 29 January 2008 [30], specifies a higher value of MRL, i.e., 0.02 mg/kg. At present, disulfoton is not approved for use in the European Union. Disulfoton is an insecticide, and it is used among other things for the control of aphids and spinning mites. This pesticide has a highly toxic effect in the case of ingestion or absorption through the peel. In the case of long-term exposure, it may have mutagenic effects and cause incorrect synthesis of DNA in fibroblasts [62,63]. The Directive of the European Commission 2003/14/EC of 10 February 2003, amending Directive 91/321/EEC on preparations for infants and related preparations [64], does not allow the use of disulfoton in agricultural raw materials for the production of infant food. The American Environmental Protection Agency placed restrictions on the use of this substance in the USA [65]. The Forest Stewardship Council put a ban on the use of this substance in forests and plantations certificated by that organisation, and classified disulfoton as "extremely hazardous" (class IA according to the World Health Organisation).

In the apples analysed in 2020, no cases of exceeded MRL were noted among the detected pesticide residues. It should be emphasised that the samples of apples from 2020 analysed in the experiment did not contain any pesticides that are not approved for use in European Union. The reduction in the diversity of the detected pesticides in 2020 compared to 2012 results from the observed trends of limitation in the use of pesticides and promotion of sustainable development.

The available publications of studies on the content of pesticides in food report infrequent instances of exceeded MRL of those agents in apples. Monitoring of pesticide residues in food conducted in Poland in the years 2004–2007 by the State Sanitary Inspection revealed exceeded MRL in 2.3% of analysed samples of apples [66]. In a study conducted by Łozowicka et al. [67] in the years 2008–2011, the presence of pesticides was noted in 11.9% (from 1.7% to 19%) of samples of apples from the regions of north-eastern Poland; pesticide levels exceeding the MRL were observed in the range from 0% to 4.4% of the analysed samples of apples. Nonrecommended substances were identified in 0.5% of analysed samples of apples (among fungicides—boscalid, among insecticides—dimethoate) [67]. The cited study demonstrated that 32.4% of fruit samples and 67.6% of samples of vegetables were free of residues of plant protection agents [67]. Nowacka et al. (2010) identified, in apples from the 2009 harvest, the following pesticides: acetamiprid (6.6%), bifenthrin (1.7%), boscalid (0.8%%), captan (22.3%), carbendazim (5.8%), chlorpyrifos (3,3%), cypermethrin (1.7%), cyprodinil (3.3%), difenoconazole (0.8%), dimethoate (0.8%), and dithiocarbamates (11.6%), but none of them were at levels exceeding the MRL value. Another study on agricultural produce from the area of south-eastern Poland, conducted in 2011 by Szpyrka et al. (2008) [68], demonstrated that 46% of analysed samples of apples contained residues of plant protection agents such as: boscalid (20%), chlorpyrifos (8%), cypermethrin (6%), cyprodinil (8%), diazinon (2%), difenoconazole (1%), dithiocarbamates (2%), fenazaquin (4%), fenitrothion (2%), flusilazole (1%), iprodione (1%), captan (46%), kresoxim-methyl (2%), pirimethanil (8%), pirimicarb (7%), and trifloxystrobin (8%). In the case of diazinon (0.02 mg/kg, MRL 0.01 mg/kg) and fenitrothion (0.01–0.03 mg/kg, MRL 0.01 mg/kg), the observed levels exceeded the MRL, and in addition, the use of those two pesticides was prohibited [68].

A 9-year experiment on apples produced in Poland (2009–2013), conducted by Łozowicka [69], revealed that in 66.5% of analysed samples, 34 pesticides were identified, among which the MRL was exceeded in 3% of the analysed samples. Furthermore, 35% of the samples contained from two to six pesticides, and one sample contained seven identified compounds. Among the 34 identified pesticides, the most frequently detected were fungicides. Samples with pesticide levels exceeding the highest permissible concentrations were more often identified in the group of insecticides.

According to the Ministry of Health Report [70], in Poland in 2017, in all 85 analysed samples of apples, the presence of a total of 29 pesticides residues were identified (out of 280 pesticides included in the analyses). In four samples, five instances of exceeded MRL values were noted. In 29% of the analysed samples no presence of pesticide residues was found [70]. In 71% of the samples residues of at least one pesticide was detected. In 39% of the sample, residues of at least two pesticides were noted. None of the analysed samples contained residues of more than six pesticides. The most frequently identified pesticides were: captan (35% of the samples, with mean concentration of 0.171 mg/kg) and boscalid (22% of the samples, with mean concentration of 0.017 mg/kg) [70]. In addition, the authors of the report noted the presence of tebuconazole in 12% of the samples, and fludioxonil in 9% of the samples [70].

A study on pesticide residues in apples grown in Greece demonstrated that 84% of analysed fruits contained pesticide residues, 55.6% of the analysed samples contained from two to four pesticides, while in 7% of the analysed samples a minimum of four of the analysed compounds were detected [71]. The most frequently detected compound was carbendazim (45.7%), followed by chlorpyrifos (44.4%). The average detected levels of concentration varied from 0.169 ppm (fluopyram) to 0.005 ppm (triazofos). Furthermore, 19 out of the 40 analysed pesticides were not identified in any of the analysed samples of apples. In several instances, the detected levels of concentration exceeded the relevant MRL values for four pesticides.

A monitoring study on apples conducted in Kuwait revealed that 90% of the analysed samples of apples contained a detectable residue of pesticides, with 80% exceeding the MRL values [72]. In five samples of the analysed apples, values above the MRL were noted for imidacloprid, in one sample the excessive concentration related to deltamethrin, and in two samples it related to malathion.

Figure 3 presents the frequency of occurrence of pesticides in the analysed samples of apples in the years of the experiment, expressed in the form of percentage share of burdened samples. In 2012, diphenylamine, a growth regulator, was detected in all samples of apples, but the level of the substance did not exceed the MRL. Furthermore, 89% of the samples of apples contained the insecticide disulfoton, and the next most frequently identified compound, at 67%, was the acaricide propargite, whereas in 2020, fungicides were detected in apple samples: boscalid with frequency of 80%, followed by pyraclostrobin and captan, detected in 60% of the analysed samples.

This report also takes into account the long-term hazard, characterised in the form of estimated daily intake of pesticide residues with apples, and indicates that the chronic exposure to captan and boscalid at the average levels observed in the analyses does not pose a threat to any consumer group. The highest estimated daily intake was noted in the case of captan, and it amounted to 213% of the value of the Acceptable Daily Intake (ADI). It was found that, in the case of children, the potential one-time (one day) intake of chlorpyrifos with a large portion of apples exceeds the value of the Acute Reference Dose (ARfD), which created a potential hazard for consumer health in that individual instance [70].

In Poland, in the period of 2014–2019, the following frequency of pesticide residue occurrence in apples was noted: from the group of fungicides, captan (88%), boscalid (38%), pyraclostrobin (28%), tebuconazole (21%), difeconazole (14%), pirymethanil (12%), fludioxonil (12%), tiofanate methyl (12%), fluopyram (10%), cyprodinil (4%), trifloxystrobin (5%), fluksapyroksad (2%), tetraconazole (2%), bupirymat (1%), izopirazam (1%), and pentiopyrad (1%); and from the insecticides, acetamiprid (58%), methoxyfenozide (30%), indoxacarb (22%), pyrimicarb (13%), chlorpyrifos methyl (10%), thiacloprid (*%), phosmet (5%), flonicamid (4%), and deltamethrin (1%) [17].

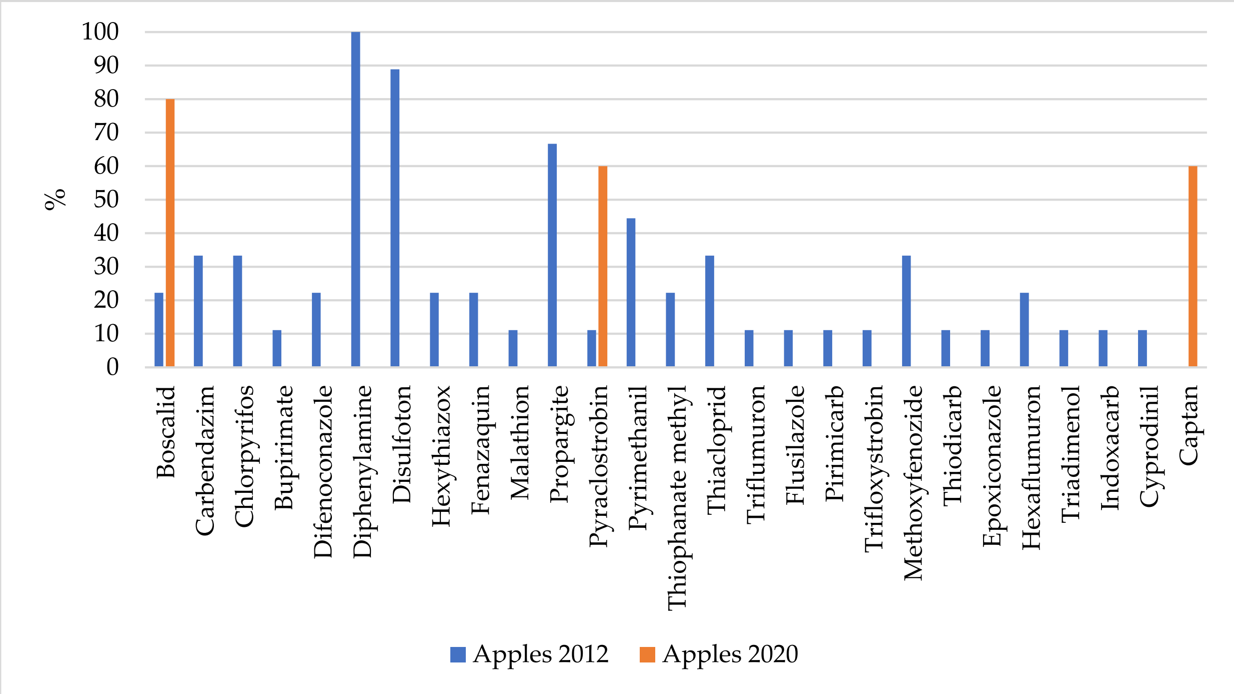

Figure 3. Frequency of occurrence of pesticides in the analysed samples of whole apples in 2012 and 2020, expressed as percentage share.

More than one pesticide was identified in many samples of analysed apples in Kuwait [72]. Co-occurrence of pesticide residues in a single sample may result from the application of various kinds of pesticides to protect fruits against various pests or diseases. In the cited study, the presence of a single compound was noted in 13 analysed samples of apples (16.25%), while the co-occurrence of two pesticides was identified in two samples (2.5%). Additionally, 16 samples (20%) contained residues of three, four, five and six pesticides, and eight samples (10%) were contaminated with residues of 7 to 14 pesticides [72]. The most frequent two-component combination of pesticides was chlorpyrifos and cypermethrin. In four-component combinations, the most frequently identified were combinations of insecticides with fungicides—boscalid, chlorpyrifos, cypermethrin and pyraclostrobin, while in samples with five pesticide residues, the most frequently identified were combinations of insecticides, fungicides and acaricides—boscalid, chlorpyrifos, cypermethrin, propargite and pyraclostrobin. The sample with the largest number of identified pesticides (as many as 14) contained three systemic insecticides (acetamiprid, boscalid, lufenuron), seven nonsystemic insecticides (chlorpyrifos, cypermethrin, deltamethrin, lambda-cyhalothrin, profenofos, triazofos, thiacloprid), two systemic fungicides (difenoconazole, pyraclostrobin), one nonsystemic fungicide (pirymethanil) and one nonsystemic acaricide (propargite) [72].

The results obtained in the study are in conformance with the results obtained by Mladenov and Shterev [73], and by Bakırcı et al. [74]. The highest levels of pesticide residues were assayed in apples from Bulgaria, produced in the conventional manner, with residues of chlorpyrifos and fenitrothion [73], and in apples from the Aegon region in Turkey, with residues of propargite and thiabendazole [74].

3.1.2. Citrus Fruits

Figures 4 and 5 present the content of identified pesticides in edible parts of citrus fruits (flesh), in samples from 2012 and 2020, respectively.

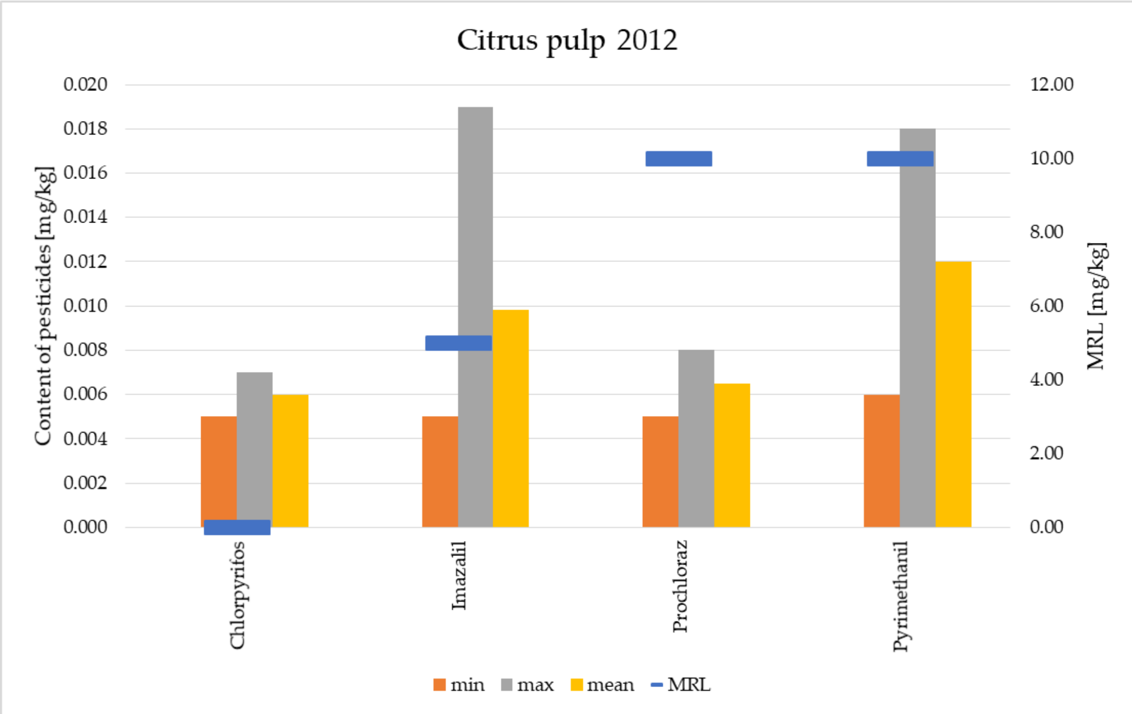

Figure 4. Content of pesticides from the edible parts of citrus fruits from the harvest of 2012.

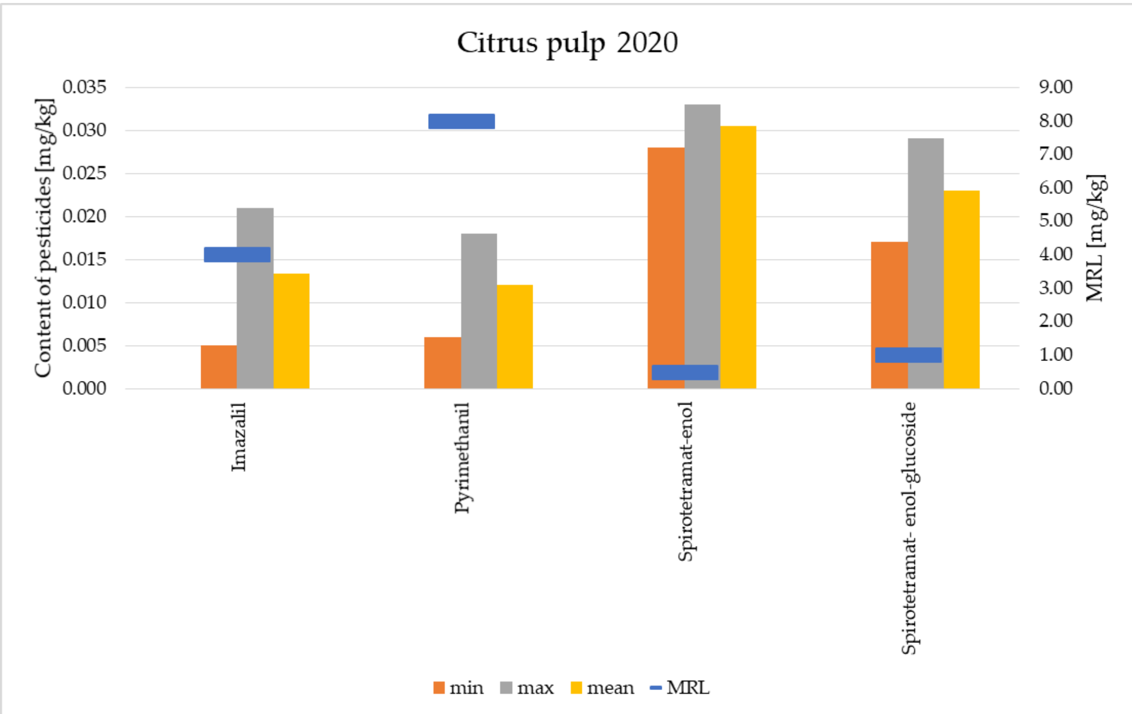

Figure 5. Content of pesticides from the edible parts of citrus fruits from the harvest of 2020.

In 2012, the presence of four pesticides were found in the flesh of citrus fruits—chlorpyrifos (insecticide), imazalil (fungicide), prochloraz (fungicide) and pyrimethanil (fungicide). In 2020, in the flesh of the analysed samples of citrus fruits the following pesticides were identified: imazalil, pyrimethanil and compounds from the spirotetramat group (insecticides), such as spirotetramat-enol and spirotetramat-enol-glucoside. In the years of the analyses, no instances of exceeded MRL values were noted for the identified pesticide residues.

Góralczyk et al. [66] conducted a study in the years 2004–2007 analysing the residues of pesticides in samples of fruits, including, among others, oranges, mandarins and nectarines. Exceeded values of MRL were observed by the authors only in the case of nectarines, and the pesticide with excessive concentration was chlorpyrifos. Thurman et al. [75] identified prochloraz and imazalil, and their primary products of degradation, in extracts of citrus fruits. Blasco et al. [76] assayed pesticide residues in oranges and tangerines from Valencia (Spain). Among 116 samples analysed by the authors, 52 contained residues of plant protection agents. The most frequently detected pesticides were the following: carbendazim, hexythiazox, metydation, imidacloprid and metiokarb, and in addition, in some samples they also identified piriproksyfen, thiabendazole, trichlorofon and imazalil. In 22 instances, the cited authors detected hexythiazox at concentrations in the range of 0.02–0.05mg/kg, and in eight samples they assayed imazalil at levels from 0.02 to 1.2 mg/kg. However, they did not observe any nonconformance with MRL in force in the territory of the EU. In a majority of cases, the samples contained residues of individual pesticides. Infrequently, two or three pesticides were identified in a sample, and in only one sample, residues of four pesticides were identified at the same time—carbendazim, hexythiazox, imazalil and metydation.

In a study on apples and lemons, Jurak et al. [77] demonstrated that 18.3% of analysed samples were contaminated with pesticides. The most frequently identified compound was izmalil, detected in 18 analysed samples (range of 0.02–4.10 mg/kg), followed by chlorpyrifos, identified in 8 samples, at concentrations from 0.03 mg/kg to 0.27 mg/kg. Imazalil constituted the highest percentage (66.6%) of pesticides identified in the analysed fruits. In eight samples of the fruits, pesticide residues were higher than the MRL and were identified as hazardous for human consumption.

A study by Calvaruso et al. [78] focused on pesticide contamination of citrus fruits (oranges, lemons and mandarins) cultivated in Italy, and demonstrated that 60% of the analysed samples of fruits contained residues of one pesticide, while in two samples (4%) the presence of two pesticides was noted. Samples of oranges contained the highest average levels of pesticides (2491 $\pm$ 1024 μg/kg), with the maximum level (4468 μg/kg) being that of imazalil, which was the most frequently identified pesticide in orange samples (83%). This confirms the extensive use of this compound in the protection of citrus fruits. The presence of imazalil was noted also in samples of mandarins, with an average level of 3841.25 $\pm$ 2145 μg/kg and a maximum of 4456 μg/kg.

A study on pesticide residues in citrus fruits (peeled grapefruit, lemon, orange and mandarin) grown in the Jordan Valley [79] revealed the presence of chlorothalonil and daminozide in the majority of the analysed samples. Their average concentrations in grapefruit, lemon, orange and mandarin exceeded the maximum residue limit (MRL) values. In some of the fruits, several other pesticides, e.g., bensulphuron methyl and demeton-S-methyl-sulphoxide, were noted at levels above the MRL.

In a study by Jurak et al. [77], pesticide residues were detected in 31 out of 200 (15.5%) samples of fruits (oranges, grapefruits, lemons, mandarins, peaches, pears, grapes) and vegetables (tomato, potato). In fruit samples, pesticide residues were found in 22 out of 120 (18.3%) analysed samples. Imazalil was identified in 18 samples (range of 0.020–4.1 mg/kg). Chlorpyrifos was detected in eight samples at concentrations from 0.030 mg/kg to 0.27 mg/kg, and foran in three samples, at low concentrations ranging from 0.011 mg/kg to 0.019 mg/kg. In a single sample, ethion was detected at a concentration of 0.27 mg/kg. Imazalil constituted the highest percentage (66.6%) of pesticides identified in the analysed fruits. In eight samples of fruits, pesticide residues exceeded the MRL values and were classified as hazardous if used for human consumption.

Figure 6 presents the frequency of occurrence of pesticides in the analysed samples of citrus fruit flesh, expressed in the form of percentage share of burdened samples.

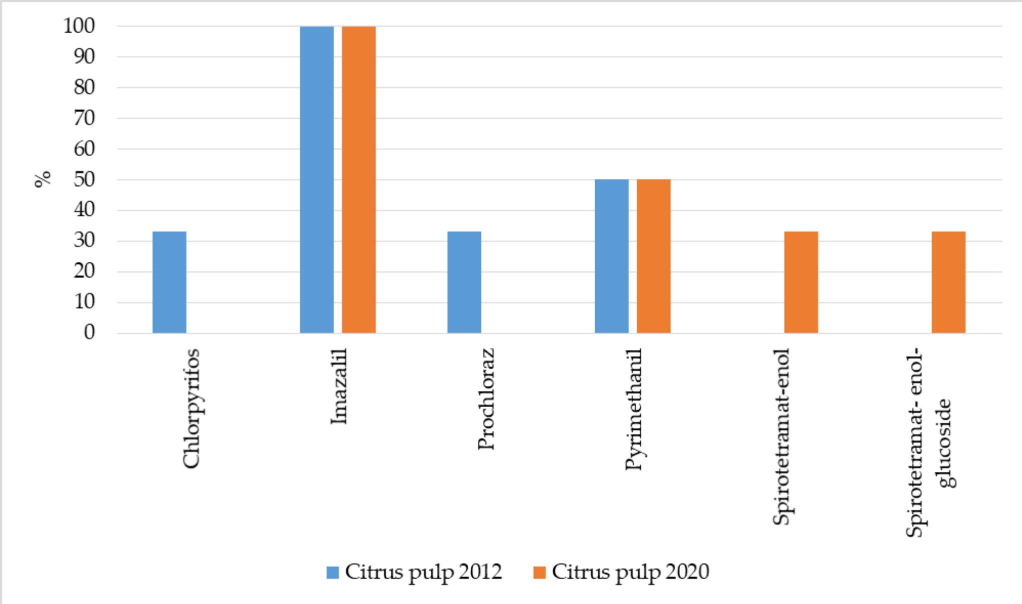

Figure 6. Frequency of occurrence of pesticides in the analysed samples of citrus flesh in 2012 and 2020, expressed as percentage share.

In 2012 and in 2020, in all analysed samples of citrus fruits, the presence of the fungicide imazalil was detected. The second most frequent pesticide in the analysed samples of citrus fruits was the fungicide pyrimethanil, whose residues were detected in 50% of the analysed samples in the years of the study. Imazalil and pyrimethanil are fungicides extensively used in agriculture, especially in the cultivation of citrus fruits. They provide surface protection of fruits, especially citrus fruits, against the growth of moulds on their surface. Unfortunately, analyses on the flesh of fruits protectively coated with those compounds prove that the fungicides penetrate into the fruits [75,76].

Comparing the results concerning the content of pesticide residues in apples and in citrus fruits, one can note that apples were characterised by a significantly greater diversity of identified pesticides, i.e., 27 and 8 identified compounds in apples, and only 4 pesticides in citrus fruits.

According to the Report of the Ministry of Health [70] in Poland in 2017, no instances of exceeded values of MRL in analysed samples of oranges were observed. No pesticide residues were found in 7% of the analysed samples [70]. In 93% of the analysed oranges, the presence of at least one pesticide was detected. In 80% of the samples, the presence of at least two pesticides was noted. None of the samples of oranges contained residues of more than nine identified pesticides. The most often identified pesticides were the following: imazalil (91% of the samples, with average concentration of 0.603 mg/kg), thiabendazole (48% of the samples, with average concentration of 0.380 mg/kg), chlorpyrifos (41% of the samples, with average concentration of 0.023 mg/kg), pyrimethanil (34% of the samples, with average concentration of 0.267 mg/kg), 2,4-D (30% of the samples, with average concentration of 0.030 mg/kg) and 2-phenylphenol (29% of the samples, with average concentration of 0.212 mg/kg), as well as carbendazim (16% of the samples), dithiocarbamates (14% of the samples), propiconazole (14% of the samples), buprofezin (13% of the samples), azoxystrobin (11% of the samples), pyraclostrobin (11% of the samples) and chlorpyrifos methyl (9%) [70].

Imazalil is a fungicide which is used mainly after the harvest, which means that it should be found on the peel of orange fruits [80]. A study conducted by Swiss researchers on citrus fruits demonstrated that imazalil was detected in 70% of cases [81]. Chlorpyrifos was identified in eight samples at concentrations from 0.030 mg/kg to 0.27 mg/kg. Chlorpyrifos is an organophosphate insecticide extensively used in agriculture. Due to the high hazard to human health, and also environmental pollution, this pesticide is prohibited from use in the EU [82]. Foran was detected in three samples at very low concentrations ranging from 0.011 mg/kg to 0.019 mg/kg. Foran is a phosphoroorganic pesticide used as an insecticide, withdrawn from use in the EU. Although it is forbidden by the EU from use in third-party countries, it can still be identified. The presence of foran was confirmed in a study conducted in India [83]. In a single sample, ethion was detected at the concentration of 0.27 mg/kg.

3.2. Effect of Peeling on the Content of Pesticides

Figures 7 and 8 present the residues of pesticides in the peels of apples from the years 2012 and 2020.

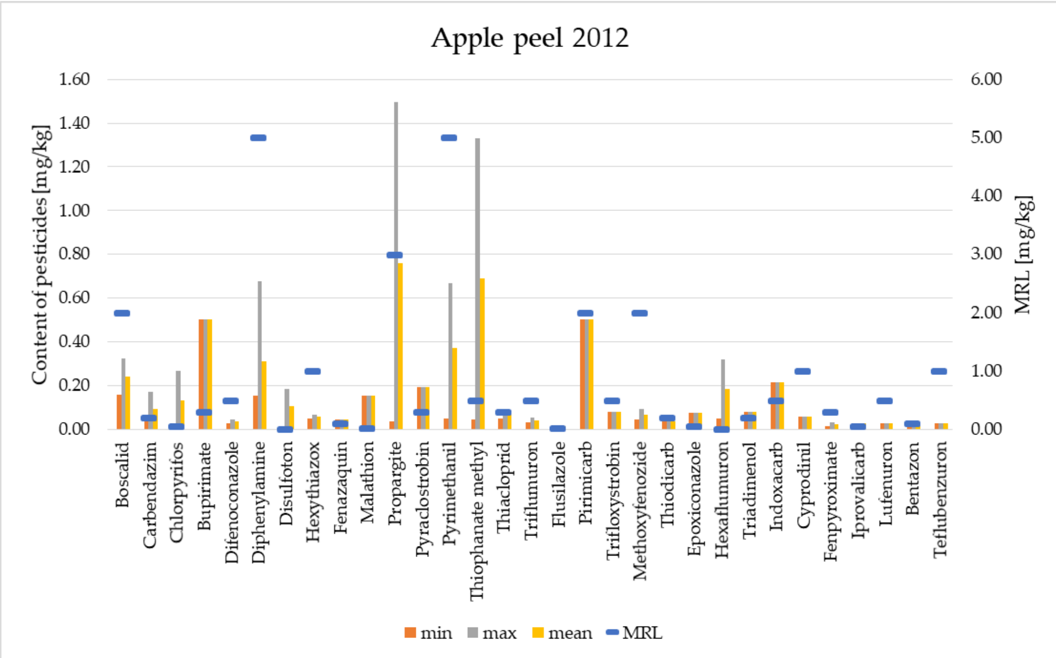

Figure 7. Content of pesticides in samples of peels of apples from the harvest of 2012.

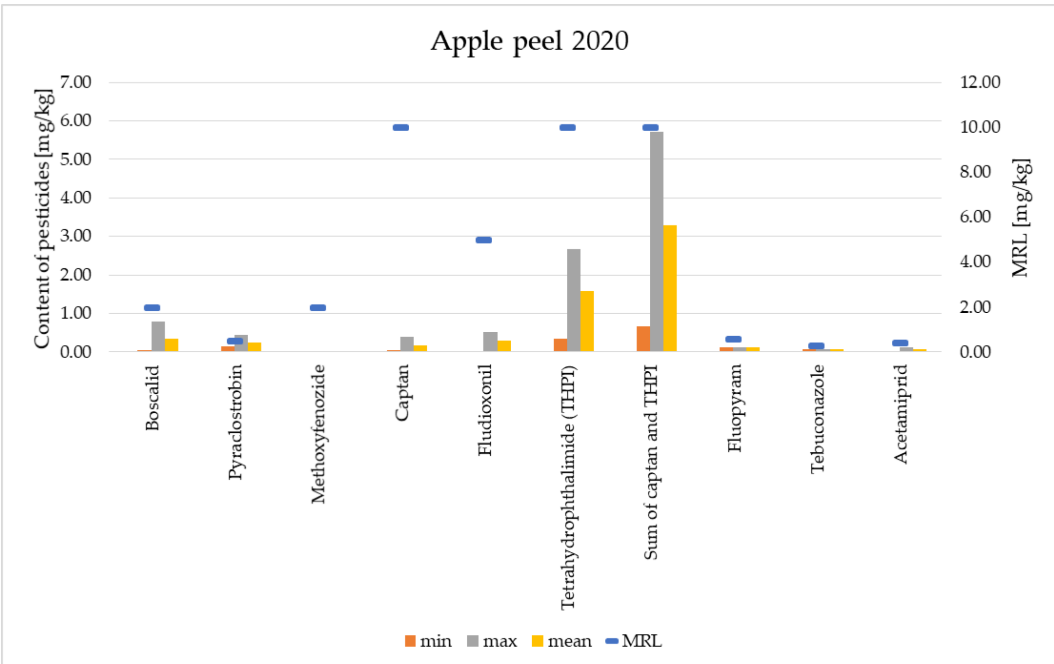

Figure 8. Content of pesticides in samples of peels of apples from the harvest of 2020.

In 2012, in apple peel, 31 compounds were identified (Figure 7, Table 1), compared to 26 compounds identified in whole apples (Figure 1). The pesticide group identified the most frequently in apple peels in 2012 was that of insecticides (52%), followed by fungicides (42%), growth regulators (6%) and herbicides (3%), whereas in 2020, residues of nine pesticides were detected in apple peels (Figure 8) compared to eight identified in whole apples (Figure 2, Table 1). Among the pesticides identified in apple peels in 2020, eight represented compounds from the group of fungicides (89%), and one represented the group of insecticides (acetamiprid, 11%).

Analysing the levels of individual pesticides in apple peels in the years 2012 and 2020, one should note that only in 2012 was the value of MRL exceeded in the cases of chlorpyrifos (MRL 0.05 mg/kg [25]) in 33% of the samples (from 0.102 mg/kg to 0.269 mg/kg), and disulfoton (MRL 0.01 mg/kg [32]) in 78% of the samples—within an 8–19-fold range compared to the allowable levels (from 0.077 mg/kg to 0.186 mg/kg). Pesticide concentrations in apple peels were significantly higher than in whole apples. It should be emphasised that the European Commission decided not to renew the approval for two active substances: chlorpyrifos and chlorpyrifos methyl, after acquiring the opinion of the European Food Safety Authority (EFSA). According to EFSA, plant protection agents containing those substances may have a negative impact on human health, and on children in particular. The fundamental argument is focused on the potential effect of the genotoxicity and developmental neurotoxicity of those chemical compounds, this being supported by epidemiological data indicating the occurrence of such effects in the population. Chlorpyrifos and chlorpyrifos methyl raise controversy not only in Europe, but also in the USA. The Californian Environmental Protection Agency arrived at similar conclusions to the EFSA, perceiving an effect of those substances on brain damage in children. While as of the 31st of December 2020, farmers in California could not possess nor use plant protection agents containing chlorpyrifos and chlorpyrifos methyl, in all other states of the USA the registration of these insecticides is currently extended till 2022. The Implementing Regulations (EU) No. 2020/18 [84] and 2020/17 [85] on the nonrenewal of the approval for the above active substances were finalised on 10 January 2020. The licence for chlorpyrifos and chlorpyrifos methyl expired on 31 January 2020. By 16 February 2020, all permits for plant protection agents containing chlorpyrifos and chlorpyrifos methyl as active substances were annulled. From that date, new batches of such insecticides could not be placed on the market, and those already on the market before 16 February 2020 could only be offered for sale until 1 April 2020. The ultimate deadline for the use of those insecticides in the territory of the European Union was the 16 April 2020. The fact that no instances of identification of chlorpyrifos in samples of apples in 2020 were noted shows that the producers of plant protection agents have complied with the above regulations.

For comparison, the content of pesticides in the flesh of fruits was determined after peeling off the peels. Figures 9 and 10 present the levels of pesticide residues in the flesh of apples in the years 2012 and 2020.

In the flesh of apples from 2012, 24 pesticides were identified, i.e., two fewer than in whole apples and seven compounds fewer than in the peels acquired from those apples (31 pesticides), whereas in the flesh of apples from 2020, only six pesticides were detected (with identification of captan metabolites), i.e., one pesticide fewer than in the whole apples and three fewer than in peels peeled off the apples. The analysis of the levels of residues of individual pesticides in apple flesh did not reveal any instances of values exceeding the MRL in the years of the study.

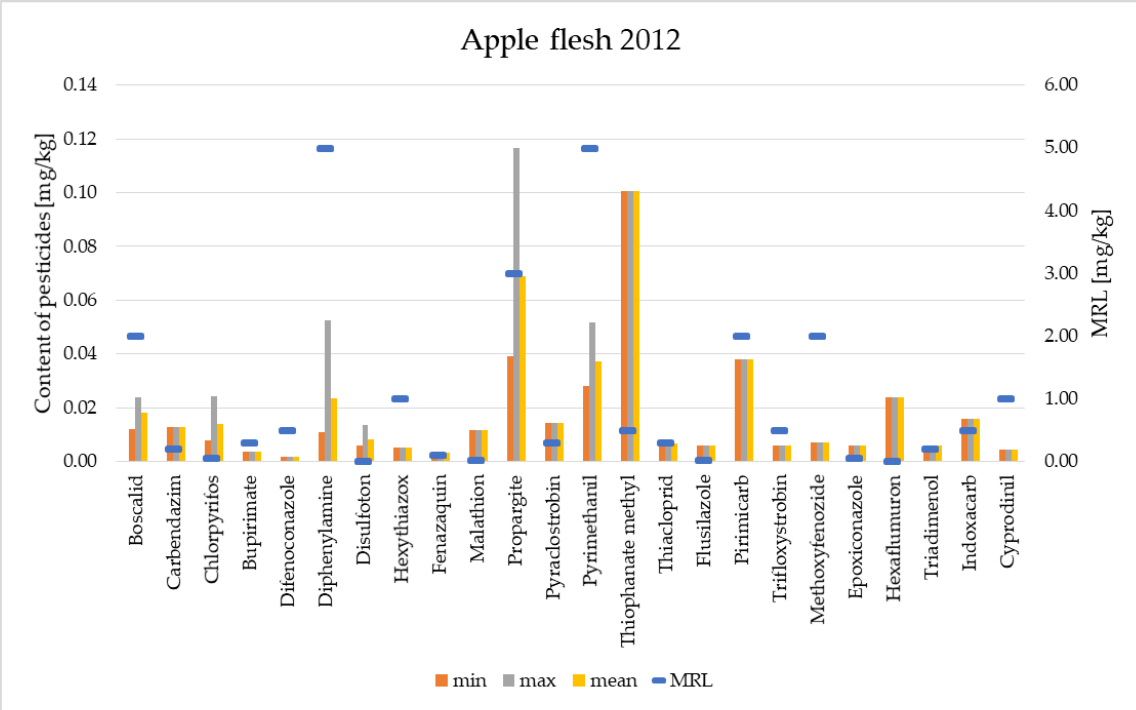

Figure 9. Content of pesticides in samples of flesh of apples from the harvest of 2012.

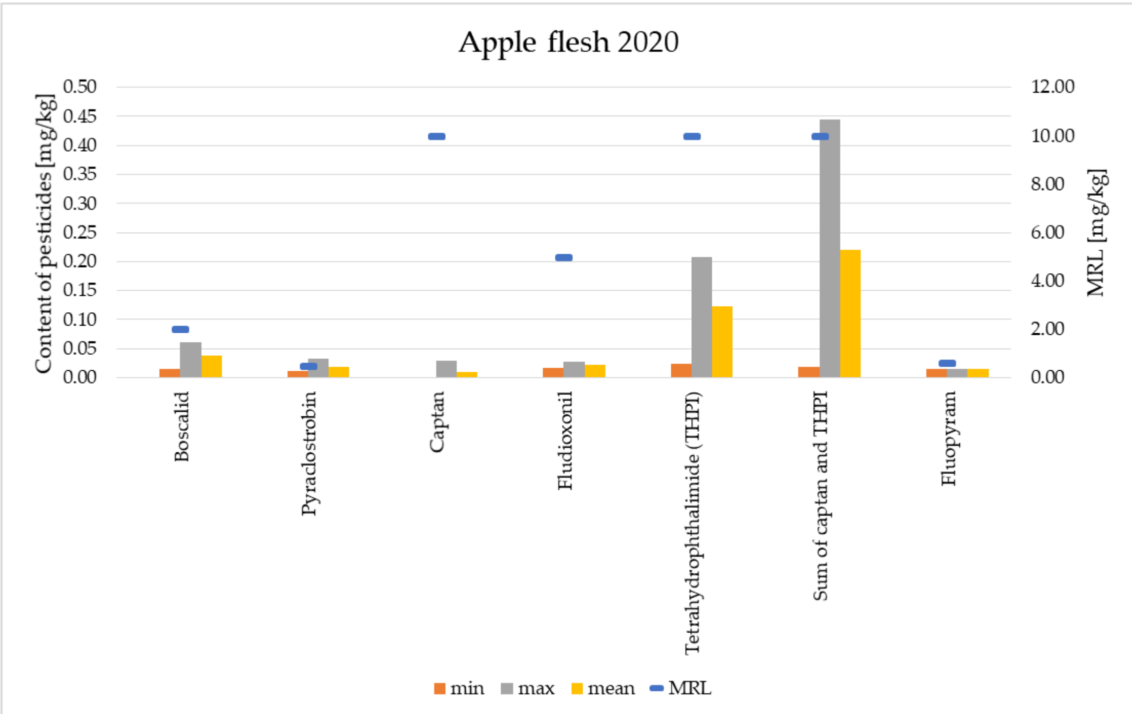

Figure 10. Content of pesticides in samples of flesh of apples from the harvest of 2020.

Accumulation of pesticides in fruits is dependent on the method of operation of plant protection agents [17]. In the case of systemic compounds, e.g., boscalid, bupirymat, cyprodinil, difenokonazol, flonicamid, fluopyram, pyraklostrobin, pirymethanil, pirymicarb, tebuconazole, tiachlopryd, tiofanate methyl, and trifloxystrobin, they penetrate into plant tissues, due to which their concentration in peeled apples is higher and more difficult to remove through the use of technological processes. In the case of nonsystemic contact operations (e.g., deltamethrin, fludioxonil and methoxyfenozide), a higher level of reduction in the levels of residues is observed in fruits with the peel removed [17].

A study conducted by Łozowicka et al. [86] on the level of contamination of apples from south Kazakhstan demonstrated that 50% of the samples contained 24 pesticides at concentrations of 0.006–0.62, 0.005–0.46 and 0.02–1.38 mg/kg, in whole apples, in the flesh and in the peel, respectively. Furthermore, 26 identified compounds had concentrations higher than the MRL, primarily the insecticide chlorpyrifos in 13 samples (MRL 0.01 mg/kg), the acaricide propargite in 10 samples (MRL 0.01 mg/kg), and triazophos (MRL 0.01 mg/kg). The compounds detected in the apples represented three groups of pesticides: acaricides (2), fungicides (8) and insecticides (14), and their concentrations ranged from 0.004 to 1.38 mg/kg in the peel, from 0.001 to 0.46 mg/kg in the flesh, and from 0.003 to 0.62 mg/kg in whole apples. The highest concentrations of the identified compounds in apple peel were noted in the case of the acaricide propargite (1.38 mg/kg), followed by the insecticides triazophos (1.25 mg/kg) and chlorpyrifos (1.03 mg/kg), whereas in samples of apple flesh, lower concentrations of pesticide residues were noted relative to the apple peels, with the highest concentrations observed in the cases of propargite (0.46 mg/kg), chlorpyrifos (0.31 mg/kg), and the fungicide boscalid (0.22 mg/kg). In whole apples, the highest concentrations were identified in the cases of propargite and chlorpyrifos, at 0.62 and 0.42 mg/kg, respectively. The next most frequently identified active substance was cypermethrin (22.5% of all analysed samples), a representative of the group of insecticides, with concentrations of 0.006–0.29 mg/kg in apple peels, 0.002–0.06 mg/kg in the flesh, and up to 0.2 mg/kg in whole apples. In 22.5% of the analysed samples, another insecticide was identified—acetamiprid—with concentrations of 0.017–0.813 mg/kg in the peel, 0.003–0.134 mg/kg in the flesh, and 0.003–0.089 mg/kg in whole apples.

In a study on four apple cultivars, Mladenova and Shtereva [73] observed significantly lower pesticide residues (below 0.05 mg/kg) in whole apples relative to the analysed samples of apple peels (0.45 mg/kg–0.77 mg/kg).

Mechanical peeling, typical of home processing, and chemical peeling, used mainly in industrial processing, are treatments which significantly contribute to a reduction in the level of pesticide residues in the flesh of fruits. Most of the pesticide residues are removed with the fruit peel [87]. In this case, the systemic operation of pesticide residues is not always correlated with lower reduction in pesticide residues through peeling [87].

Our experiment demonstrated a significant effect of peeling on the content of pesticides in apples (Figures 11 and 12). Peeling allowed us to reduce the levels of pesticides, expressed in %, within the range from 24% (carbendazim) to 100% (triflumuron, thiodicarb, tebuconazole).

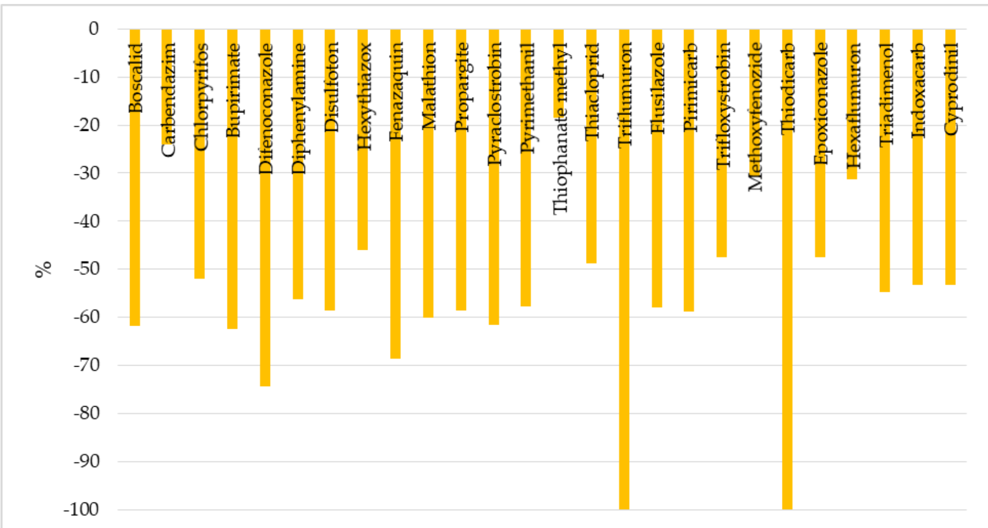

Figure 11. Percentage reduction in pesticide residues in apples as a result of peeling in 2012.

Figure 12. Percentage reduction in pesticide residues in apples as a result of peeling in 2020.

Figures 13 and 14 illustrate the content of pesticide residues in the peel of citrus fruits from the harvests of 2012 and 2020. Table 2 presents data concerning the occurrence of individual pesticides in the analysed citrus fruits, taking into account the MRL and LOQ values.

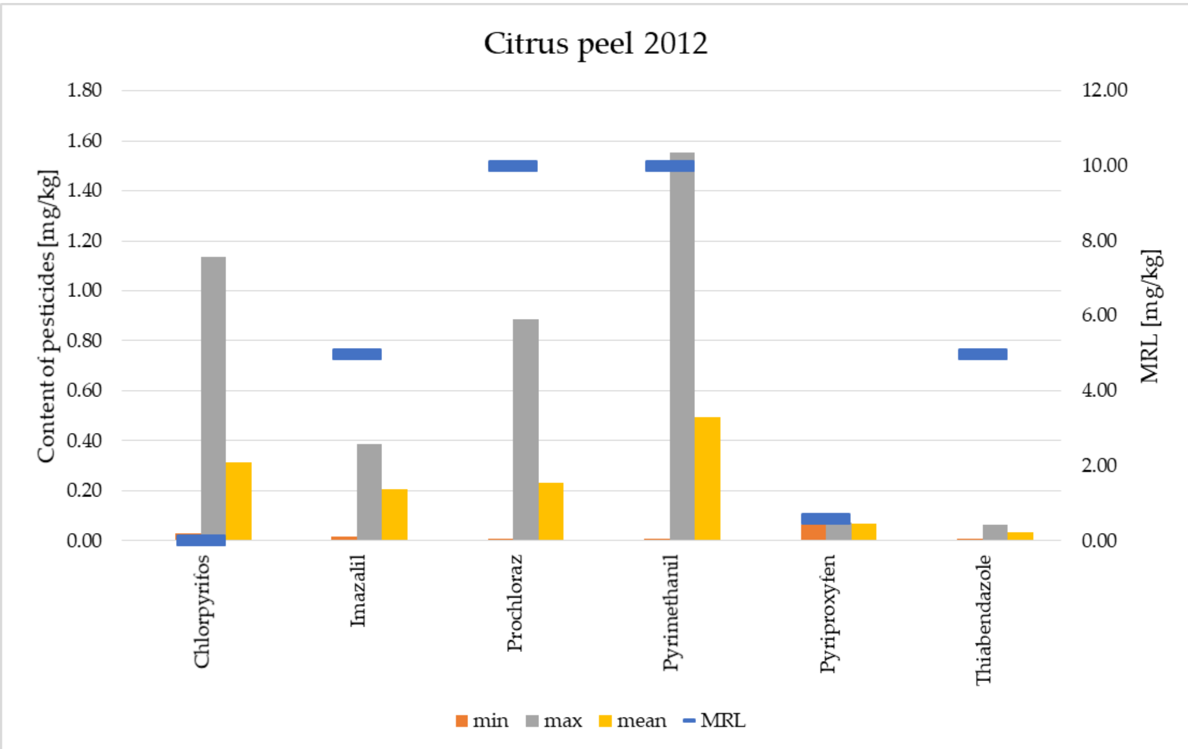

Figure 13. Content of pesticides in peels of citrus fruits from the harvest of 2012.

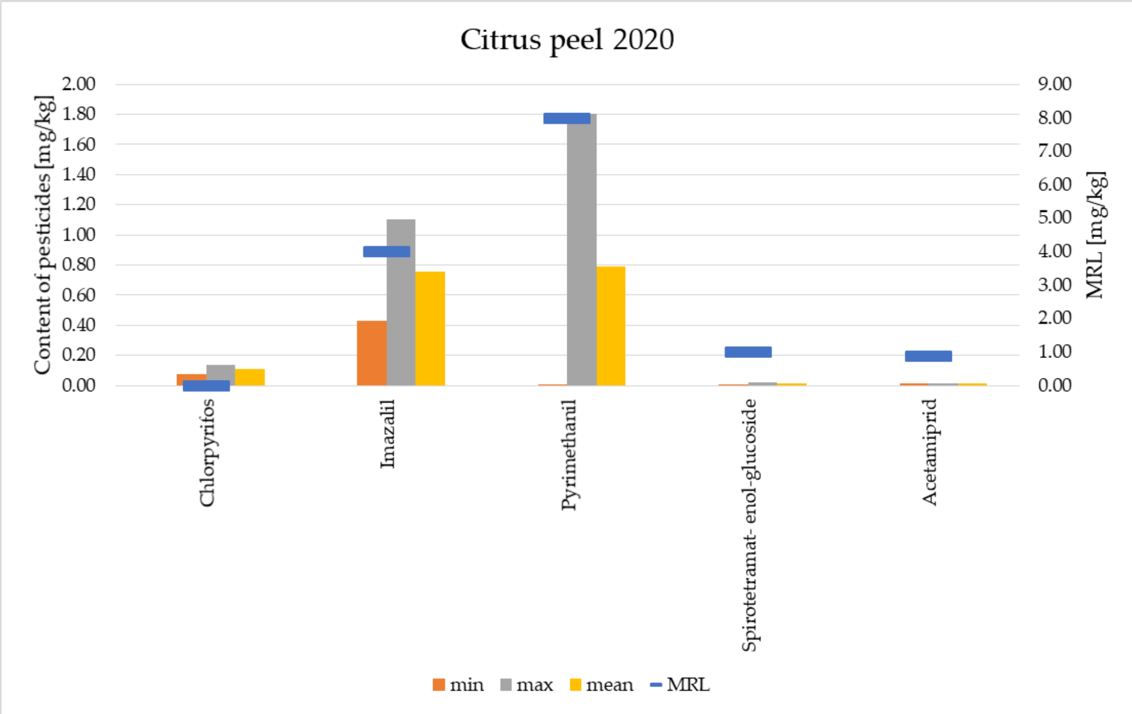

Figure 14. Content of pesticides in peels of citrus fruits from the harvest of 2020.

Table 2. Occurrence of individual pesticides in citrus fruits and the values of MRL.

Pesticide	Group	Range of Pesticides Concentration				MRL		LOQ mg/kg
		Citrus Pulp		Citrus Peels				
		2012	2020	2012	2020	2012	2020	
chlorpyrifos	A, I	<LOQ–0.0070 c	<LOQ d	0.0300–1.1350 a	<LOQ–0.1380 b	0.01 mg/kg [25]	not approved 0.01 mg/kg [26]	0.0001
imazalil	F	0.0050–0.0190 c	0.0050–0.0210 c	0.0160–0.3860 b	0.4320–1.10 a	5mg/kg [44]	grapefruits and oranges 4 mg/kg lemons 5 mg/kg [54]	0.0005
prochloraz	F	<LOQ–0.0080 b	<LOQ c	<LOQ–0.8840 a	<LOQ c	10 mg/kg [51]	0.03 mg/kg [88]	0.0002
pyrimethanil	F	<LOQ–0.0180 c	<LOQ–0.0180 c	0.0090–1.5550 b	0.0100–1.8000 a	10 mg/kg [39]	8 mg/kg [38]	0.0005
pyriproxyfen	I	<LOQ b	<LOQ b	<LOQ–0.0720 a	<LOQ b	0.6 mg/kg [25]	0.6 mg/kg [54]	0.0001
thiabendazole	F	<LOQ b	<LOQ b	<LOQ–0.0640 a	<LOQ b	5 mg/kg [30]	7 mg/kg [89]	0.0001
spirotetramat-enol spirotetramat-enol-glucoside	F	- -	<LOQ–0.0330 a <LOQ–0.0290 a	- -	<LOQ b 0.0090–0.0240 a		0.5 mg/kg [90] 1 mg/kg (expressed as spirotetramat) [56]	0.01
acetamiprid	I	-	<LOQ b	-	<LOQ–0.0170 a	1 mg/kg [39]	0.9 mg/kg [59]	0.0001
hexythiazox	A, I	-	<LOQ b	-	<LOQ–0.0110 a	1 mg/kg [33]	1 mg/kg [33]	0.0001

F—fungicide, I—insecticide, A—acaricide, LOQ—limit of quantification. a, b, c, . . . —values designated with the same letters in line do not significantly differ at 5% error (Duncan's test).

Peels of citrus fruits are not used for direct consumption, with the exception of lemons, whose peel has some spice value. Peels of citrus fruits can be processed and used as an additive in confectionery products, or they can be raw materials for the production of essential oils.

In 2012, in the peel of citrus fruits, two more pesticides were identified relative to the results for the fruit flesh, i.e., pyriproxyfen (insecticide) and thiabendazole (fungicide). If we compare the normative requirements relating to the peels of citrus fruits to those relating to citrus fruits, instances of levels in excess of the MRL value (0.01 mg/kg [25]) were noted only in the case of chlorpyrifos in all of the analysed samples, with detected concentrations in the range from 0.030 mg/kg to 0.045 mg/kg (lemons), 0.040 mg/kg to 0.096 mg/kg (oranges), and from 0.541 mg/kg to 1.135 mg/kg (grapefruits). In samples of the peel of citrus fruits from 2020, six pesticides were identified, i.e., chlorpyrifos, imazalil, pyrimethanil, spirotetramat-enol-glucoside, acetamiprid and hexythiazox. As in 2012, in the samples of citrus fruit peels from the year 2020, excessive levels of pesticides were noted only in the case of chlorpyrifos—in two samples of grapefruit peels the values were 0.074 mg/kg and 0.138 mg/kg, and in one sample of orange peels the assayed value was 0.12 mg/kg (the MRL was unchanged relative to earlier regulations and equalled 0.01 mg/kg [26]).

The levels of pesticide residues in the flesh of citrus fruits have been presented in the preceding subchapter (Figures 4 and 5).

The results clearly indicate a high accumulation of pesticides in the peels of citrus fruits. In the case of the flesh of citrus fruits, residues of plant protection agents were identified in minimal amounts and never exceeded the valid MRL values. Similar conclusions were formulated by Ortelli et al. [81] following an experiment on various citrus fruits: lemons, oranges, mandarins, grapefruits, limes, pomelo and kumquats. Among 240 analysed samples, 207 samples contained residues of plant protection agents such as imazalil, thiabendazole and prochloraz, in 70%, 36% and 11% of the samples, respectively. In addition, those authors observed notable levels of chlorpyrifos, hexythiazox, methidathion and tebufenpyrad. They also noted considerable concentrations of pesticides in the peels of the analysed fruits. On the basis of an experiment on lemons and oranges, they concluded that fungicides applied after the harvest of the fruits accumulated in 85–90% in the fruit peel.

In samples of lemon peels, the presence of fenhexamid was detected, at an average concentration of 30.25 $\pm$ 18.34 mg/kg and a maximum of 66 mg/kg [78]. Analysis of the specific parts of the fruits of orange and mandarin revealed significant differences in the level of imazalil. In addition, differences in the level of fenhexamid were noted in the analysed parts of lemon fruit samples. In the analysed samples of orange, 6% of imazalil migrated from the peel to the flesh, while in the case of mandarin that value was as low as 1.6%. In the case of samples of mandarins, 16% of carbendazim migrated from the peel to the flesh, and in the case of samples of lemons 25% of fenhexamid penetrated from the peel to the flesh. The relatively low rate of penetration of fungicides to the flesh probably resulted from the physicochemical properties of the analytes under examination and of the peel of the analysed fruit samples. In reality, the levels of both fungicides were considerably higher in the peels than in the whole fruits. In view of the above, and of the lack of knowledge on the origin of the products, it is not recommended to use peels of citrus fruits for the preparation of meals or drinks. In actual life, the peels of citrus fruits are often used in the preparation of liqueurs, jams, or pastry.

In a study on oranges, Li et al. [91] demonstrated a significantly higher content of pesticides in the peels compared to the flesh (7.5–17.9%), with the exception of prochloraz, whose content in the flesh of the fruits was 65.4%. Peeling resulted in a significant reduction in the level of residues of pesticides in the fruit flesh [91].

Impregnation of citrus fruits through immersion in a solution containing fungicides is extensively applied to prevent losses during fruit storage and distribution [81]. That procedure was the cause of residue levels exceeding the MRL demonstrated by the cited authors in the case of chlorpyrifos and imazalil. In the case of imazalil, the highest concentration was ca. 1.1 mg/kg, and in the case of chlorpyrifos ca. 1.6 mg/kg, i.e., considerably higher than in the peels of citrus fruits analysed in this study. Such a situation poses a potential hazard to the consumer, especially when also fruit peels are consumed, e.g., candied peel of lemons, oranges and grapefruits (commonly used in the confectionery industry).

Another important aspect is the total content of all pesticides, which can have a synergistic effect on human health, hence the importance of limitation of the use of pesticides. This is reflected in the observed trend, especially in the case of apples, where in 2020 the levels and numbers of identified pesticides were significantly lower compared to the year 2012.

3.3. Effect of Conventional Washing of Fruits on the Content of Pesticides

Figures 15 and 16 illustrate the levels of pesticides in apples from the harvests of 2012 and 2020, respectively, subjected to conventional washing with tap water. The experiment demonstrated that conventional washing of fruits does not cause any reduction of the number of identified pesticides, i.e., 26 pesticides were detected in 2012 and 7 pesticides in samples of apples from 2020. Analysing the levels of residues of the particular pesticides, no significant differences were noted between samples of apples washed with water and those which were not subjected to the process of washing.

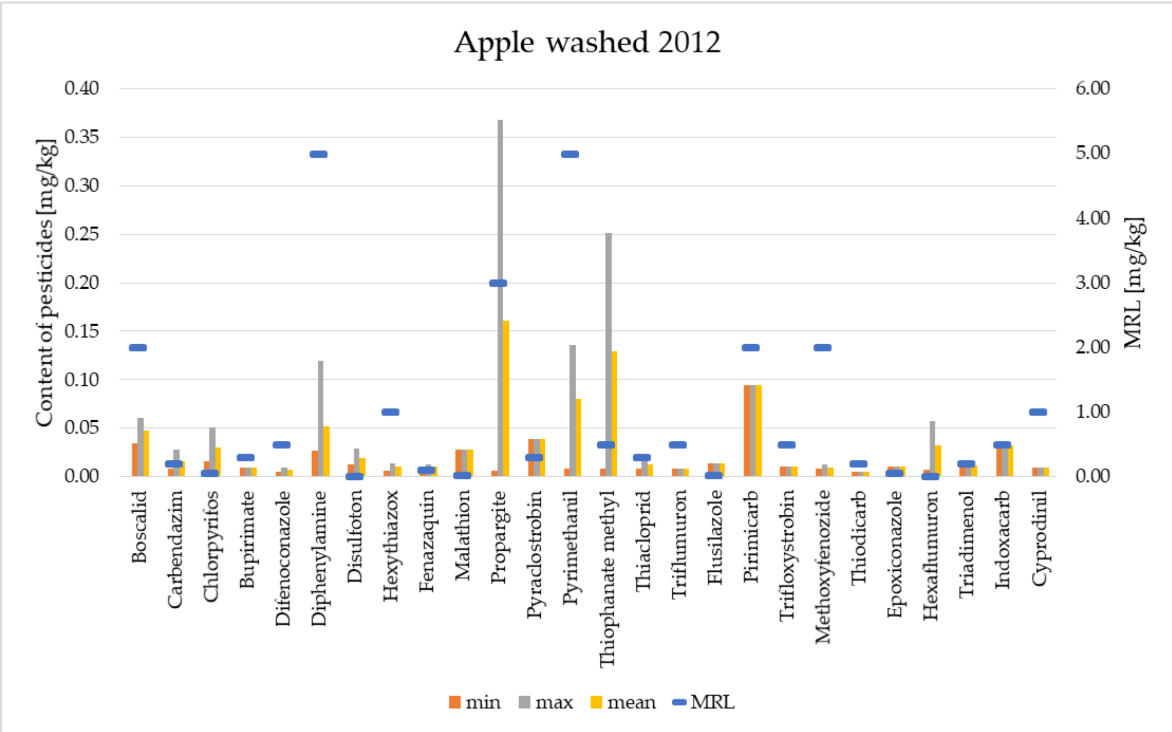

Figure 15. Content of pesticides in samples of apples washed with water (2012).

Yang et al. [18] also demonstrated a low effectiveness of washing apples for 2 minutes in tap water in the removal of residues of pesticides that had been introduced as standard into the analysed samples.

In a study conducted in 2015 in Iran, it was demonstrated that 67% of the analysed samples of apples contained pesticide residues [92]. The cited report describes the effect of washing and peeling on the reduction in the levels of residues of three pesticides (diazinon, chlorpyrifos and abamectin). Washing with water for 3 minutes caused a reduction in the content of the pesticides in the apples by as little as 17%, while peeling reduced the content of diazinon and chlorpyrifos by an average of 80% [92].

Sekachaie et al. demonstrated that washing with water, with water with a detergent, and peeling, resulted in a reduction in the level of malathion by 34.4%, 55.8%, 60.6% and 74.7%, respectively [93]. The washing of agricultural produce with water or with other disinfecting solutions causes the removal of only a part of pesticide residues from the surface, and also contributes to a reduction in further penetration of pesticides from the peel to the flesh of fruits and vegetables. Literature data on the effect of washing on the level of pesticide residues in fruits and vegetables are inconsistent, and all of them indicated an absence of correlation between solubility in water and the reduction in pesticides after washing [87].

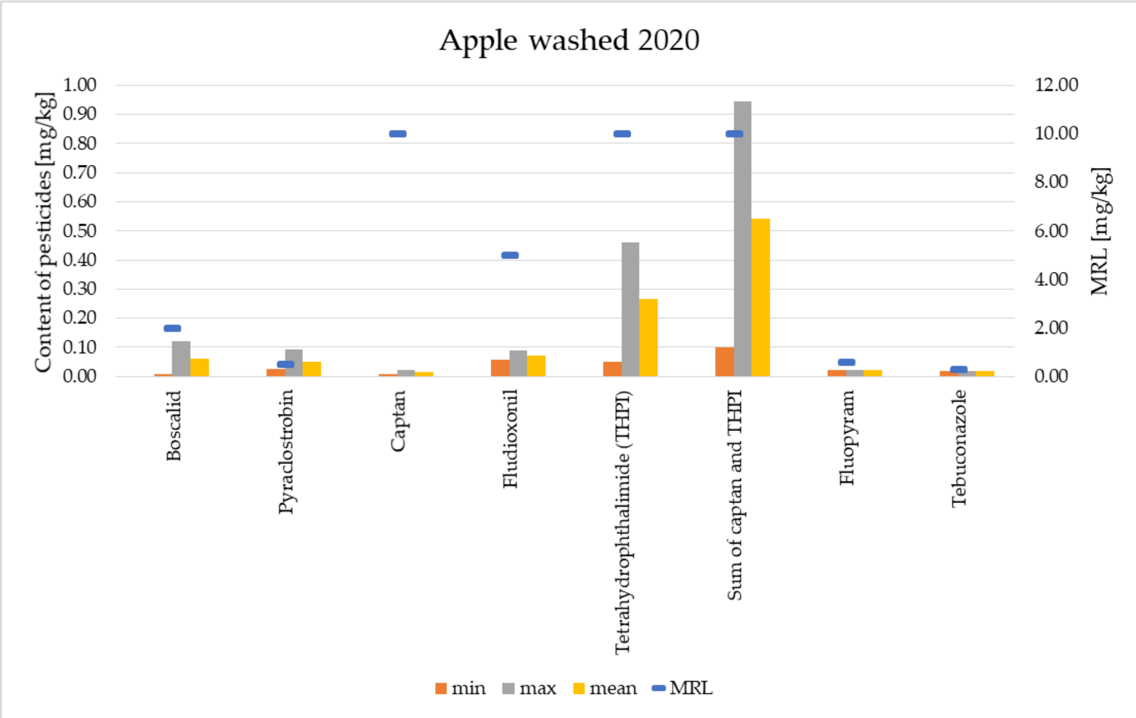

Figure 16. Content of pesticides in samples of apples washed with water (2020).

4. Conclusions

Research reports published to date indicate the significance of studies concerned with the estimation of the occurrence of residues of plant protection agents in fruits in terms of compliance with the relevant regulations. Systematic research on the toxicity of pesticides contributes to continuous and dynamic verification of approvals for the use of plant protection agents in cultivations, and to the updating of the values of MRL. The results of studies show that the ban on the use of certain pesticides leads to the expected reduction in their residues in apple and citrus fruit crops. In addition, the growing consumer awareness in the area of food safety and on the subject of the effect of pesticides on the environment results in the producers' interest in the limitation of the use of such chemicals, and in the search for alternative methods of crops cultivation, such as integrated production or organic farming. Additionally, important factors in reducing the level of pesticide residues are the simple procedures of washing and peeling, which can be performed in every household. The research carried out indicates that in the fruit of apples grown in Poland, as well as imported citrus fruits over the period of 8 years, one can clearly observe a limitation in the use of pesticides, which results in higher quality of the most popular fruits in the diet. In addition, in recent years there were no instances of identification of pesticides banned from use, which shows that producers comply with the current regulations. The simple culinary treatment of conventional peeling can largely reduce the level of pesticide residues in the edible parts of fruits.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app12031417/s1, Table S1: List of pesticides determined in the positive ionization mode; Table S2: List of pesticides determined in the negative ionization mode.

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