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Research Paper

Pesticides Load Analysis of Sewage Water and Agriculture Soil of Mangalagiri Region by Liquid Chromatography – Mass Spectroscopy (LC-MS) Analysis

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A B S T R A C T

Pesticides are the chemical compounds which are used in agricultural practice and plays an essential role in the exponential increase in crop yield through preventing diseases due to pest control. The excess use of fertilizer, nutrient content may increase toxicity through trace elements present in the environment. The aim of the present was to evaluate the pesticide by liquid chromatography – mass spectroscopy (LC-MS) in the soil and water samples collected from Mangalagiri, Guntur district of Andhra Pradesh, India. The water samples were collected in the dark bottle. The soil sample was collected from Agriculture land. The present study can be concluded as the Mangalagiri region of Guntur district showed pesticide tresses in the soil and water samples. Around 50 different types of pesticides were detected in the soil sample, whereas around 16 different pesticides were present in the water sample.

***Key words*:** Pollution, Pesticides, Liquid chromatography – mass spectroscopy (LC-MS)

Environmental pollution arises due to pesticides and heavy metal ions and it is exerted harmful effect due to bioaccumulation. Therefore, it shows toxic effects on plant, and animal and human health [1]. Pesticides are the chemical compounds which are used in agricultural practice, for the protection the crops from the pests, diseases and rodents. The pesticide use in agriculture plays an essential role in the exponential increase in crop yield through preventing diseases due to pest control [2]. The excess use of fertilizer and nutrient increases toxicity through trace elements present in the environment. This leads to elevate soil pollution due to effluents and incorrect disposal of solid waste like as domestic, industrial and rural [3], [4], [5]. The major source of heavy metal ions from industrial and consumer waste [6]. The heavy metal ions are found in aquatic plants with high amount due to the accumulation [7].

Earlier studies were determined the hazardous effects of pesticide exposure [8]. The World Health Organization and the UN Environment study reveal that, each year, three million farm workers suffered from severe pesticide poisoning [9]. In India, around 51% food material is contaminated with residues in comparison to 21% worldwide, among them 20% were above MRL prescribed by FAO standards [10]. Due to the lack of awareness of consequences of pesticide contamination in the food, could be one of the reasons for the increased incidences of cancers in the developing world. Besides the affecting human health, excess use of pesticides adversely affects the natural biodiversity [11].

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Estimation of pesticides and heavy metal ions from water is highly difficult to estimate due to lower concentration [12]. High Performance Liquid Chromatography (HPLC) method is commonly used for estimation of polar, nonvolatile and thermally labile [13]. Although, many authors studied multi-residue pesticide analysis of water by HPLC [14] and Gas Chromatography (GC) techniques [15], the use of advanced techniques like LC-MS and GC-MS are more easy, suitable and precise, and exhibits accuracy and confirmatory results [16], [17]. In view of the above-stated ill effects associated with plant and human health the present study on pesticide detection was undertaken in Mangalagiri, Andhra Pradesh, India. The soil and water sample were collected from the study location and evaluated by liquid chromatography – mass spectroscopy (LC-MS).

MATERIALS AND METHODS

The present study was conducted in the Mangalagiri region, Guntur district of Andhra Pradesh, India (16.4346° N 80.5662° E).

Sample collection

The water and soil samples were from study region. The water samples were collected in the dark bottle. The soil sample was collected from Agriculture land. Both the samples (water and soil) were protected from the sunlight and preserved for the further study.

Pesticide detection by liquid chromatography-Mass spectrometry (LC-MS)

Sample preparation: Collected surface water and agriculture soil sample from Mangalagiri were analyzed for its

pesticide content using liquid chromatography – mass spectroscopy (LC-MS). For standard solution, about 10ppt standard pesticide was mixed in the distal water (10ml) and soil (10gm). In the above samples, 10ml ethyl acetate and 5gm sodium sulphate were added. The blank reaction lacks standard pesticide. Blank and reactions were centrifuged at 5000rpm, for 10 min at room temperature (Remi C-852 Laboratory Centrifuges). In the supernatant, 250mg of sodium sulphate and 50mg primary-secondary amine were added an allow to stand for 10 min. The samples were evaporated using a nitrogen evaporator (10min, 10-15 Pas). The samples were preserved till the analysis. Various concentrations (1, 5, 10, 15 and 100 ppb) standard solution was prepared for analysis. For the blank, methanol was used.

LC-MS procedure: Before loading samples to the LC-MS, 1ml methanol and 0.1% acetic acid were added to each sample. Liquid Chromatography-Mass Spectrometry (LC-MS) Procedure was done as per the protocol described by the [18]. The LC-MS was consisting of Binary LC 1260 coupled with Agilent 6540 UHD QTOF mass spectrometer (Agilent Technologies, California, United States). Samples (2µl) were injected onto a column Inertsil ODS 4, 3um × 2.1mm × 150mm (Make - GL Sciences Inc.) at 40°C. The chromatographic conditions were as follows: A mobile phase was a combination of two solvent viz. methanol with 0.1% Formic acid (solvent A) and water with 0.1% Formic acid and 10mM Ammonium acetate (solvent B). The flow rate was 0.5ml/min. The amount of pesticide was calculated based on the reference values.

The area was calculated using following formula (Trapezoidal Rule)

$$AUC_{1\text{-}2} = [(Cp_1 + Cp_2) / 2] \times (t_2 - t_1)$$

Where,

1 and 2: x intercept points of the curve; Cp_1: x intercept of point 1; Cp_2: x intercept of point 2; t_1: time at point 1; t_2: time at point 2

RESULTS AND DISCUSSION

In the present study, soil and water samples from the Mangalagiri, Andra Pradesh, India were collected. The samples were evaluated for the presence of pesticides by liquid chromatography-mass spectrometry (LC-MS).

Detection of pesticides in standard

The (Table 1, Fig 1) depicts the LC-MS analysis of standard pesticide chromatography and their compounds list, respectively. The standard compounds showed a concentration range from 0.01 to 0.2 mg/kg with retention time varies from 1.3 to 13.4. Around 50 compounds were considered in the present study.

Table 1 List of pesticides detected in the standard by LC-MS

Compound	Retention time	Area	Con (mg/kg)
Methamidophos	1.356	4499923	0.107
Acephate	1.538	3132653	0.105
Omethoate	1.785	4160593	0.103
Dinotefuran	2.059	3194030	0.105
Carbofuran-3-Hydroxy	2.555	1382233	0.010
Thiamethoxam	2.912	9150858	0.102
Methomyl	2.920	7513619	0.105
Benomyl-I	3.272	27548953	0.103
Carbendazim	3.272	39429908	0.103
Chlothianidin	3.342	2589261	0.098
Acetamiprid	3.516	18655625	0.104
Dimethoate	3.499	16691051	0.102
Thiachloprid	3.724	19664812	0.102
Thiophenate methyl	4.210	21677575	0.101
Carbaryl	4.601	1746422	0.101
Carboxin	4.731	20023533	0.104
Thiodicarb	5.073	15116876	0.101
Atrazin	5.388	48325844	0.102
Carbofuran	5.442	37386553	0.010
Azoxystrobin	6.462	34797261	0.102
Dimethomorph-2	6.999	8751814	0.099
Triadimefon	7.153	6349985	0.101
Dimethomorph-1	7.004	8473765	0.102
Dodine	9.614	7764335	0.103
Bifenazate	7.896	7556299	0.102
Cyazofamide	8.780	2591825	0.103
Malathion	7.315	4539936	0.104
Tetraconazol	8.127	10228554	0.102
Abamectin	13.300	309473	0.106
Phenthoate	9.673	3123161	0.101
Diflubenzuron	9.069	1196232	0.099
Penconazol	9.366	5619382	0.103
S-metolachlor	8.665	16421650	0.103
Metolachlor	8.661	21480459	0.104
Emamectin Benzoate	11.413	7029964	0.103
Edifenfos	9.864	14637280	0.101
Benalaxyl-M	9.845	2157990	0.101
Etrimfos	10.049	6340037	0.107
Benalaxyl	9.844	29579183	0.103
Diazinon	10.179	19943565	0.104
Hexaconazole	9.852	17201174	0.097
Bitertanol	10.313	11578250	0.099
Difenconazole	10.679	28302342	0.102
Thiobencarb	10.576	2553488	0.101
Allethrin	11.824	1158537	0.102
Buprofezin	11.620	10121875	0.104
Dithianon	3.341	88303	0.100
Fipronil	8.883	742577	0.103
Fipronil Sulfone	9.814	314553	0.103
Hexythiazox	12.188	1845317	0.103

Detection of pesticides from the soil sample

The soil sample collected from Mangalagiri was evaluated by LC-MS analysis. (Fig 2) depicts LS-MS chromatogram of soil sample. Total 39 pesticides were detected from the sample.

Some pesticides such as carboxin, carbofuran, diflubenzuron, malathion, dimethomorph-2, carbofuran, emamectin benzoate, dithianon and fipronil sulfone were absent in the soil sample. The list of detected pesticides represents in Table 2. The standard compounds showed a concentration range from 0.002 to 0.013 mg/kg with retention time varies from 1.3 to 13.4. All detected pesticides showed lower concentration as compared to their respective standard pesticide concentration.

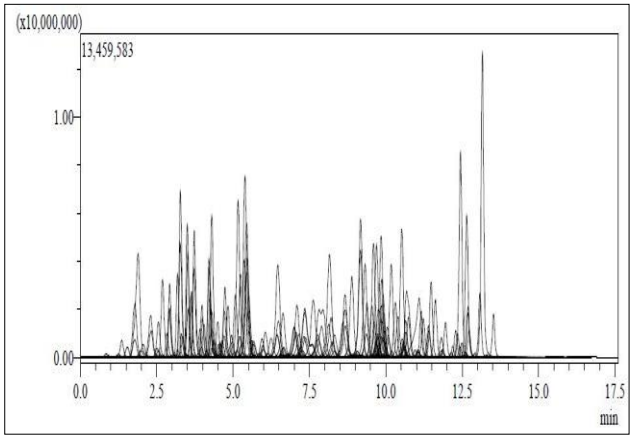

Fig 1 LC-MS chromatogram of standard pesticides

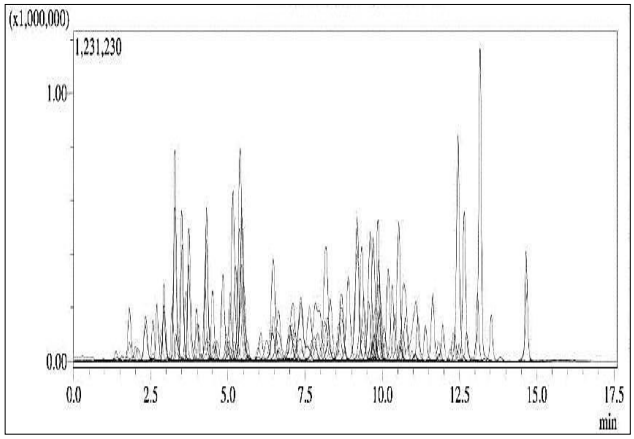

Fig 2 LC-MS chromatogram of soil sample

Table 2 List of pesticides detected in the soil sample by LC-MS

Compound	Retention time	Area	Con (mg/kg)
Methamidophos	1.372	173739	0.004
Acephate	1.563	100550	0.003
Omethoate	1.810	281405	0.007
Dinotefuran	2.091	217919	0.007
Carbofuran-3-Hydroxy	2.566	110522	0.001
Thiamethoxam	2.918	847137	0.010
Methomyl	2.930	629384	0.009
Benomyl-I	3.280	3151048	0.012
Carbendazim	3.279	4430612	0.012
Chlothianidin	3.347	294542	0.011
Acetamiprid	3.516	1780155	0.010
Dimethoate	3.500	1514564	0.009
Thiachloprid	3.724	1858992	0.010
Thiophenate methyl	4.211	1960	0.000
Carbaryl	4.598	157080	0.009
Carboxin	--	--	--
Thiodicarb	5.073	1419977	0.010
Atrazin	5.392	4976548	0.011
Carbofuran	--	--	--
Azoxystrobin	6.467	3377302	0.010
Dimethomorph-2	--	--	--
Triadimefon	7.164	648431	0.010
Dimethomorph-1	7.017	888438	0.011
Dodine	9.633	373112	0.005
Bifenazate	7.906	579991	0.008
Cyazofamide	8.786	227672	0.010
Malathion	--	--	--
Tetraconazol	8.143	1112115	0.011
Abamectin	13.293	22956	0.009
Phenthoate	9.686	292355	0.010
Diflubenzuron	--	--	--
Penconazol	9.373	611429	0.012
S-metolachlor	8.679	1653515	0.011
Metolachlor	8.670	2094441	0.010
Emamectin Benzoate	--	--	--
Edifenfos	9.877	1707500	0.012
Benalaxyl-M	9.850	203350	0.010
Etrimfos	10.055	602515	0.011
Benalaxyl	9.857	3038746	0.011
Diazinon	10.184	1776737	0.009
Hexaconazole	9.867	2092492	0.012
Bitertanol	10.323	1373854	0.012
Difenconazole	10.697	3042270	0.011
Thiobencarb	10.593	273833	0.011
Allethrin	11.828	89118	0.009
Buprofezin	11.630	1001011	0.010
Dithianon	--	--	--
Fipronil	8.875	67237	0.010
Fipronil Sulfone	--	--	--
Hexythiazox	12.198	176162	0.010

The consumption rate of the pesticide is increasing at 8% every year. Nearly 70% of all pesticides consumed in India belong to the category of the agro-chemicals banned or restricted in a number of countries [19]. 28 domestic samples were studied from the different regions of Hyderabad and Andhra Pradesh for presence of pesticides and reported the presence of lindane, DDT, α-endosulfan and β-endosulfan pesticides in the water samples [20]. Various reports were published about the residual effect on the vegetables from the Andhra Pradesh [21]. The presence of monocrotophos, chlorpyrifos, cypermethrin and endosulfan pesticides on high concentration in the vegetables like Brinjal, cucumber, okra, ridge gourd and tomato [22]. While, the surface water showed the presence of pesticide residues below maximum residue limit (MRLs).

Pesticide detection from water sample

The water sample collected from Mangalagiri was subjected to liquid Chromatography – Mass Spectroscopy (LC-MS) analysis for pesticide detection. The LC-MS chromatogram is shown in the (Fig 3).

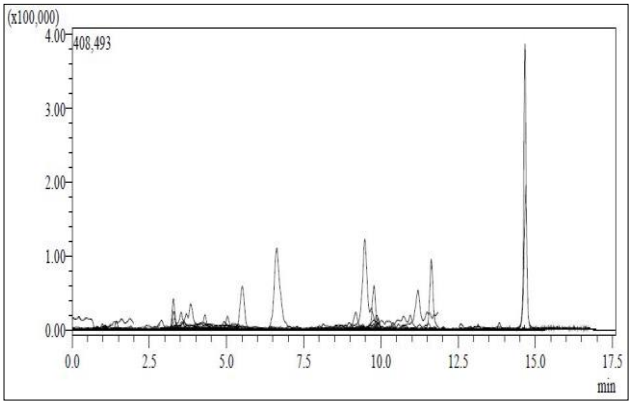

Fig 3 LC-MS chromatogram of water sample

About 16 pesticides were detected from the water sample. The lists of pesticides are given in the (Table 3). In the standard around 50 pesticides were detected. However, some of them were not found in the collected water sample.

Those pesticides were omethoate, dinotefuran, thiamethoxam, chlothianidin, dimethoate, thiachloprid, carboxin, atrazin, carbofuran, dimethomorph-2, triadimefon, dimethomorph-1, dodine, bifenazate, cyazofamide, abamectin, phenthoate, diflubenzuron, penconazol, s-metolachlor, metolachlor, emamectin benzoate, edifenfos, benalaxyl-m, etrimfos, benalaxyl, diazinon, bitertanol, thiobencarb, allethrin, dithianon, fipronil, fipronil sulfone and hexythiazox. Collected water samples showed 16 pesticides and their quantity ranges from 0.001 to 0.004 mg/kg (ppm).

Ample number of studies has been published about the pesticide detection in the water from various parts of India such as Delhi [23], [24], [25], Haryana [26], South India [28], Uttar Pradesh [29], Uttarakhand [30], Maharashtra [31], Gujrat [32], Andhra Pradesh [33] etc. The study reported from the Guntur region of Andhra Pradesh states the lower awareness about pesticide pollution in cotton and chilli crops in the farmers [34]. The LC-MS analysis of the spices samples from the Guntur region of Andhra Pradesh reported that the pesticides such as acephate, carbofuran, chlorpyriphos, endosulfan, monochrotophos and quinolphos were present below detectable limit (BDL) i.e., very low amount [35]. Our report is accordance with this report. Similar pesticides were also present in our water sample.

Table 3 List of pesticides detected from the water sample collected from Mangalagiri.

Sr. No.	Compound	Retention time	Area	Con. (mg/kg)
1.	Methamidophos	1.596	6068	0.000
2.	Acephate	1.658	9980	0.000
3.	Carbofuran-3-Hydroxy	2.661	1269	-0.000
4.	Methomyl	2.578	5970	0.000
5.	Benomyl-I	3.280	146816	0.001
6.	Carbendazim	3.276	228350	0.001
7.	Acetamiprid	3.516	11036	0.000
8.	Thiophenate methyl	4.347	3559	-0.000
9.	Carbaryl	4.602	1977	0.000
10.	Thiodicarb	4.998	1848	0.000
11.	Azoxystrobin	6.444	45404	0.000
12.	Malathion	7.289	6521	0.001
13.	Tetraconazol	8.184	14566	0.000
14.	Hexaconazole	9.856	139413	0.001
15.	Difenconazole	10.729	46742	0.000
16.	Buprofezin	11.635	415101	0.004

CONCLUSIONS

The present study can be concluded as the Mangalagiri region of Guntur district showed pesticide tresses in the soil and water samples. Around 50 different types of pesticides were detected in the soil sample, whereas around 16 different pesticides were present in the water sample. However, the concentration of pesticides was found to be below maximum residue limit as compared to standard values. There is an urgent need to educate the farmers to use integrated pest management practices to minimize the use of chemical pesticides in order to avoid adverse effect of pesticide residues in food chain.

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