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Extraction and Clean-up Techniques for Selective Isolation of Atrazine and Ametryn Residues from Soil Samples of Wonji Shoa Sugar Cane Plantation Farm

M. Sc Graduate Project

Submitted to

Department of Chemistry

By Bezuayehu Tadesse

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DEDICATION

I dedicate this project work to my son, Betselot.

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ACKNOWLEDGMENTS

I am most grateful to my project advisor, Dr. Negussie Megersa, for his guidance and invaluable advice. My admiration also goes to my co-advisors Mr. Tarekegn Birhanu and Mr. Ahmed Hussen for their advice and providing literatures related to my project, and their closer assistance in the experimental activities.

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LISTS OF ABBREVIATIONS

HA**….** Hydroxy atrazine (6-ethylamino-2- hydroxy-4- isopropylamino 1, 3, 5 triazine.

DEA…. desethylatrazine (6-amino-4-isopropylamino-2-chloro-1,3,5-triazine)

DIA ….desisopropylatrazine (6-amino-4-ethylamino-2-chloro-1,3,5 triazine)

HPLC …High Performance Liquid Chromatography

2,4-D…. 2,4- Dichlorophenoxyaceticacid

SOM ….Soil Organic Matter

ASE …. Accelerated Solvent Extraction

% …….Percentage

IUPAC... International Union of Pure and Applied Chemistry

SSE….Solvent Shake Extraction

SOX….Soxhlet Extraction

ABSTRACT

In this research work atrazine and ametryn, which are applied as mixture known by brand name of i W to sugar cane plantation farms were extracted employing two conventional extraction techniques, namely, solvent shake and soxhlet. The extraction was carried out using soil samples collected from wonji shoa sugar cane plantation farm, following the standard procedures. Prior to the analyte extraction, moisture contents of the soil samples were determined. It has been found that for all samples the moisture content values range between 4.6-7.7 %. Thus, the soil samples were over dried and extraction of the target compounds was performed. Moreover, since the colour of the resulting extracts was light yellow and appeared to be turbid, further cleaning has been arranged. Clean-up of all extracts were therefore utilizing florisil packed in a glass column and topped with anhydrous sodium sulphate. The eluate from the glass column, which was nearly colourless, and assumed to be free of particulate matters was evaporated to dryness and reconstituted in 1.5 ml acetonitrile. It was then stored in a refrigerator, at 4 ^{o}C until used for final analysis by HPLC.

Wonji Shoa sugar industry is one of the biggest sugar industries in Ethiopia. A mixture of atrazine and ametryn (Gesapax Comb 500FW) is frequently applied herbicides in wonji shoa sugar cane plantation. Soxhlet extraction and solvent shake extraction methodology have been applied for extracting atrazine and ametryn from the collected soil samples. Five soil samples were collected from different fields for selective isolation of atrazine and ametryn.

1. INTRODUCTION

1.1 Pesticides

Pesticides are group of human-made or natural chemical compounds that are used to kill unwanted pests and other living things (insects, fungi and weeds). During the past years, many works have emphasized ecological damage produced because of the widespread use of agricultural pesticides. There mobility through air and water, their accumulation and their biomagnifications constitute a real risk to human health, wildlife and the environment [1-4].

Chemicals like pesticides once introduced to soil environments can behave in different ways: (i) Existing in an unchanged form for a long time being sorbed by different soil compartments, (ii) transforming to other compounds, sometimes even more dangerous than the original ones, and (iii) leaching from soil and pollute the surface or ground water [5].

Many processes affect the fate of pesticides in the environment. These processes include adsorption, transfer, breakdown and degradation. Transfer includes processes that move the pesticide away from the target site. These include volatilization, runoff, leaching and absorption.

Historically, in agricultural terms, a persistent pesticide was considered to be one whose residues remained in the soil in significant quantities after application until the next growing season or until the sowing or planting of the following crop [6-7].

The overall loss of pesticide in soil generally follows first-order kinetics, which can be expressed mathematically as follows:

$$C = C_o e^{-kt}$$

Where C is concentration of the chemical (mg/kg soil) at any time *t*; C_o is initial concentration of the chemical (mg/kg soil); *k* is the first-order decay rate constant per day; *t* is elapsed time after application day [8-9].

1.2 Herbicides

Herbicides are chemicals with a capacity to kill certain plants selectively or non-selectively and a heterogeneous group of chemicals used against weeds in intensive farming [10-11]. Herbicide, in the broadest sense, is defined as any chemical compound that is capable of either killing or severely injuring weeds and may thus be used for elimination of unwanted plant growth. Herbicides show a wide range of beneficial effects such as improving plant health, food supply, and etc .The removal of weeds can also save livestock from poisonous plants. According to the general definition, a weed is any plant- either a wild or cultivated variety- that is undesired in that particular place (plant out of place). On railway tracks, industrial sites, airports, paths and open spaces the entire vegetation can be regarded as weeds [12].

A weed is a plant that does more harm than good. Weeds may cause reduced yield and financial losses in crop production by:

i. competing for plant food, water and light.
ii. interfering with harvest and so increasing harvest costs.
iii. lowering the quality of grain through contamination.
iv. reducing quality indirectly as a result of harmful diseases and pests associated with weeds.

1.2.1 Classification of Herbicides

1.2.1.1 The total or non- selective herbicides

Non-selective herbicides are intended to kill all vegetation present. Their use in agriculture is obviously limited and is restricted to removing unwanted vegetation around buildings and on roads.

1.2.1.2 Selective herbicides

Selective herbicides are used extensively in agriculture. They are intended to suppress or kill some plants without seriously affecting others, thus showing “selectivity” between the weeds (plant out of place) and the crop (the plants that are required).

Herbicides are applied either to the foliage of weeds or to the soil. Foliage treatments are sub divided according to the way they affect the weeds. A contact herbicide treatment destroys plant tissues only by external contact and does not move far from the point of application. In contrast, growth regulators herbicides treatments, after entry, move within the plant to affect tissues away from the point of application, either in the shoots or in the roots.

Herbicides applied to the soil or residual herbicides are absorbed by the roots of seedling weeds as they germinate or by the roots of established weeds and may move within the plant to affect tissues away from the point of contact.

Herbicides are applied to soil to manage weed. While it is desirable for the chemicals to control weeds during the season of application, it is not desirable for them to persist and affect subsequent crop growth. The length of time a herbicide remains active in soil is called “soil persistence,” or “soil residual life”.

1.3 Factors Affecting Herbicide Persistence

Several factors affect the persistence of a given herbicide. The main three factors are discussed below:

1.3.1. Soil Factors

Soil factors affecting herbicide persistence include soil composition, soil chemistry and microbial activity. Soil composition is a physical factor determined by the relative amounts of sand, silt, and clay in the soil, as well as by the organic-matter contenst. An important chemical property of soil that can influence herbicide persistence is pH. The microbial aspects of the soil environment include the types and abundance of soil microorganisms present in the soil.

1.3.2. Climatic Factors

The climatic variables involved in the herbicide breakdown are moisture, temperature and sunlight. Herbicides degradation rates generally increase as temperature and soil moisture increase, because both chemical and microbial decomposition rates increase with higher temperatures and moisture levels.

1.3.3. Herbicide Properties

A herbicide’s chemical properties affect its persistence. These properties include water solubility, vapor pressure and the molecule’s susceptibility to chemical or microbial alteration or degradation.

Leaching is one mechanism responsible for herbicide dissipation. The solubility of herbicide in water helps for determining its leaching potential. Leaching occurs when a herbicide is dissolved in water and moves down through the soil profile. Herbicide that readily leach may be carried away from crop and weed germination zones.

A herbicide chemical structure dictates how the herbicide will degrade in the soil. Some herbicides are rapidly decomposed by microorganism if the right kind and number are present and if soil conditions are favorable for their growth. The chemical structure of 2,4 D, for example, allows microbes quickly to detoxify the molecule into inactive metabolites, whereas atrazine is not prone to microbial attack and hence degradation is slower [13].

Table 1. Herbicides Most Commonly Used in Sugar Cane Plantation Farms in Ethiopia [14].

Trade Name	**Common Name**	**Approved Use**
Gesapax Combi 500 FW	Ametryn + Atrazine	Various weed species in sugarcane
Gesaprim 500 FW	Atrazine	Complex weeds in maize and sorghum
Dicopur 720 SL	2,4-D	Broadleaf weeds in cereals
U-46 D Fluid 72 % EC	2,4-D	Broadleaf weeds in cereals and sugarcane

1.4 Symmetrical-Triazine (*s*-Triazine) Herbicides

Triazine is the chemical species of six-membered heterocyclic ring compound with three nitrogens replacing carbon-hydrogen units in the benzene ring structure. The names of the three isomers indicate which of the carbon-hydrogen units on the benzene ring position of the molecule have been replaced by nitrogens, called 1,2,3-triazine, 1,2,4-triazine, and 1,3,5-triazine respectively. Symmetrical 1, 3, 5-triazine is the common one.

Symmetrical or 1,3,5-Triazines are used as selective pre-and post emergency maize, corn, sorghum, sugar cane, pineapple and many others. Their extensive use causes pollution of soil and consequently pollution of food and drinking water by the pesticide unmetabolized forms and their degradation products (metabolites) [15-16]. Derivatives of 1, 3, 5-triazine herbicides are applied worldwide and proved to be rather stable in the environment. Of all the triazines, atrazine is the most prominent derivative [17]. The interaction of triazines with the soil depends on the physical, chemical and biological

characteristics of both the pesticides and soil. Bioavailability is, for instance, influenced by the presence of soil organic matter (SOM), capable of binding pesticides [18-19].

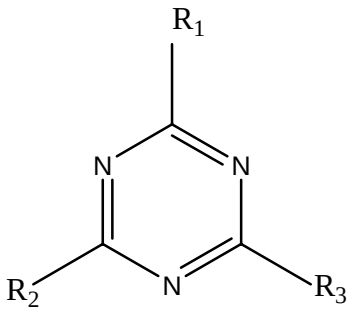


Figure1. Group Structure of *s*- triazine

1.4.1. Chemical and Physical Properties of Atrazine and Ametryn

1.4.1.1. Atrazine

Atrazine has been used since 1958 as a pre-and post-emergent herbicide [20]. Atrazine is selective triazine herbicides commonly used to control broadleaf and grassy weeds in agricultural lands and on non-cropped industrial lands [8, 21, 22, 23]. Atrazine is almost non-volatile and its half-life in neutral condition is about 200 days but varies from 4-57 weeks depending on various environmental factors like pH, moisture content, temperature and microbial activity [24]. Atrazine is used in combination with many other herbicides [25].

It is persistent in both soil and groundwater. The fate of atrazine in soil depends upon several factors including sorption to soil component, up take by plants, transport via runoff and leaching, biodegradation, photodegradation, volatilization, and chemical degradation. Adsorption of atrazine to soil components is key processes that can control several other factors. For example, the herbicide is being shielded from biodegradation, possibly through sorption to natural organic matter. Adsorption directly influences various processes, such as leaching and degradation [26]. Atrazine can be adsorbed by clay minerals and organic matter. The pH of the medium has a large effect on atrazine.

As the solution pH decreases, atrazine adsorption by clay minerals increases because more of the atrazine molecules are in the protonated form [27].

Corn and other crops are able to take up atrazine, but decompose it enzymatically before herbicidal effects occur. In addition, atrazine has recently been reported to have long-term reproductive and endocrine-disrupting effects [20, 28].

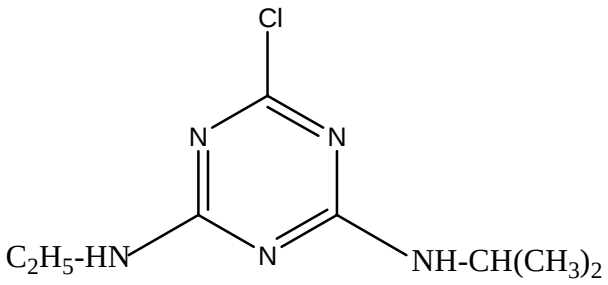


Figure 2. 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine. **(ATRAZINE)**

Table 2. Physical Properties and Nomenclature of Atrazine [29-31].

Nomenclature	**IUPAC**	2-chloro-4-ethylamino-6-isopropylamino-s-triazine
	Common name	Atrazine
Molecular Formula		$C_8H_{14}ClN_5$
Form		Colorless Powder
Melting Point		175-177 ^oC
Solubility		In water: 33 mg/L
(20-25 ^oC)		In ethyl acetate: 24 g/L
		In acetone: 31 g/L
Stability		Relatively stable in neutral, weakly acidic and weakly alkaline media
Density		1.187 gm/cm^3
Vapour Pressure at 20 ^oC		3 x10^{-7} mm Hg
pK_a (21 ^oC)		1.7
Molecular Weight		215.7

1.4.1.2 Ametryn

Ametryn, a member of the triazine family, is a herbicide which inhibits photosynthesis and other enzymatic processes. It is used to control broadleaf weeds and annual grasses in pineapple, sugarcane and bananas. Symptoms of acute exposure to high doses include nausea, vomiting, diarrhea, muscle weakness and salivation. Ametryn is moderately irritating to the eyes, skin, and respiratory tract.

The half-life of ametryn in soil is 70 to 250 days, depending on the soil type and weather conditions. Loss from the soil is principally by microbial degradation. Ametryn moves both vertically and laterally in soil due to its high water solubility. Since it is persistent, it may leach as a result of high rainfall, floods, and furrow irrigation.

Ametryn is a colorless crystal. It is non-corrosive and is stable in neutral and weak acid or basic solutions. However, it is hydrolyzed by strongly acidic or basic solutions into an inactive derivative. UV light also decomposes it slowly [32].

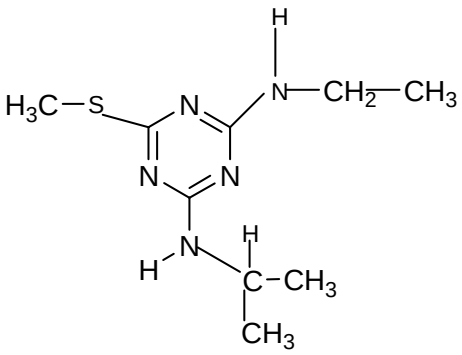


Figure 3. 2- methylthio-4-ethylamino-6-isopropylamino-s-triazine [33].

(AMETRYN)

Table. 3 Physical Properties and Nomenclature of Ametryn **[29-31].**

Nomenclature	**IUPAC**	2-Methylthio-4-ethylamino-6-isopropylamino-s-triazine
	Common name	Ametryn
Molecular Formula		$C_9H_{17}N_5S$
Form		White Powder
Melting Point		86.3-87 ^oC
Solubility (20-25 ^oC)		In water: 200 mg/L
		In acetone: 610 g/L
		In methanol: 510 g/L
Stability		Stable in neutral, weakly acidic and weakly basic media. Hydrolysed by strong acids and alkalis to the herbicidally-in active 2-hydroxy derivatives
Density		1.18 g/cm^3
Vapour Pressure at 20 ^oC		8.4 x10^{-7} mm Hg
pK_a (21 ^oC)		4.1
Molecular Weight		227.3

1.5 Synthesis of Atrazine and Ametryn

The starting material for the manufacture of atrazine and ametryn are cyanuric chloride, the trimerization product from cyanogen chloride. The stepwise replacement of two of the three chlorine atoms by identical or different C_2-C_4 – alkylamino residues leads to the first generation of triazine herbicides, of which atrazine is the most important [12].

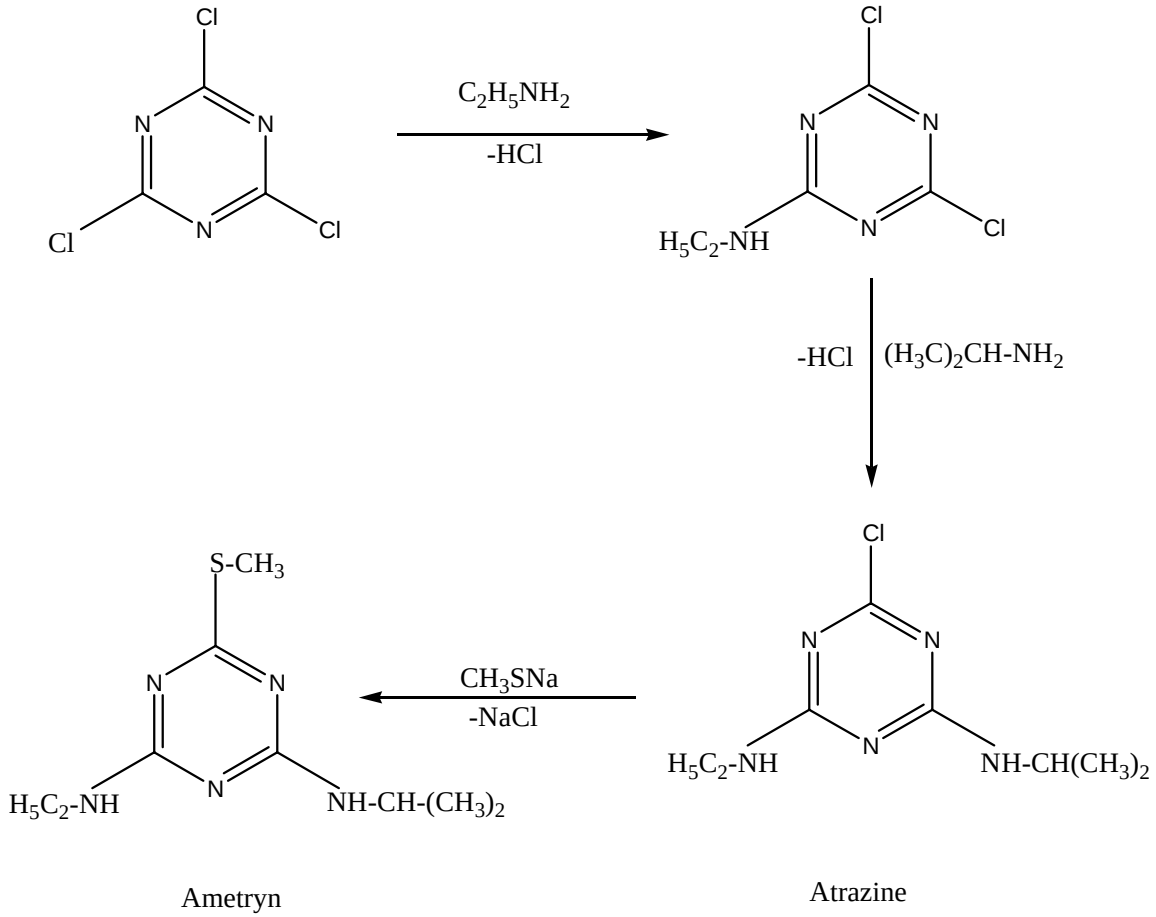
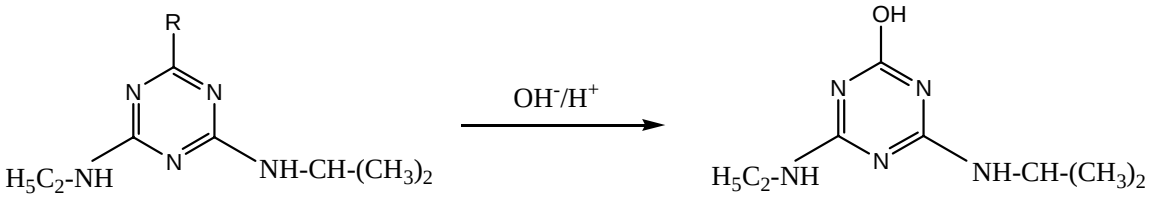


Figure 4. Synthesis Pathways of Atrazine and Ametryn [12]

1.6 Degradation of Herbicides in Soil

The heterogeneous system of soil is capable of executing diversified reactions of physical, chemical and biochemical nature. While the physical properties of the soil responsible for the adsorption and translocation of a herbicides can be measured relatively easily, the separate analysis of its chemical and biochemical degradative capacity is problematic [31].

1.6.1 Degradation Reaction of the C-2 Substituents



R= Cl, SCH_3 **HA**

Figure 5. Pathways of Atrazine and Ametryn Degradation Products [31]

In natural soils, detoxification of atrazine occurs principally by chemical hydrolysis in the 2-position. Chemical hydrolysis of atrazine produces hydroxyatrazine in strongly acidic or basic solution. Alkaline hydrolysis likely involves direct neucleophilic displacement of Cl^- from 2-position of atrazine by OH^- whereas acid hydrolysis may results from protonation of a ring or chain nitrogen atom followed by cleavage of the C-Cl bond by water. That may be the cause of more rapid hydrolysis of atrazine in alkaline medium than in acidic medium [34].

Several factors were reported to affect this detoxication reaction. Increasing temperature, moisture, low pH and high organic matter content favored the hydrolysis of chloro triazines.

1.6.2 Degradation Reaction of N-Dealkylation

Pesticides used in agriculture and forestry are mainly adsorbed and degraded in top soil. Microbial degradation of atrazine in the soil yields deethylatrazine and deisopropylatrazine, These microbial degradation products have relatively high mobility and potential to contaminant ground water. In addition, photodegradation of atrazine on the soil surface produces DEA and diaminochlorotriazine [35].

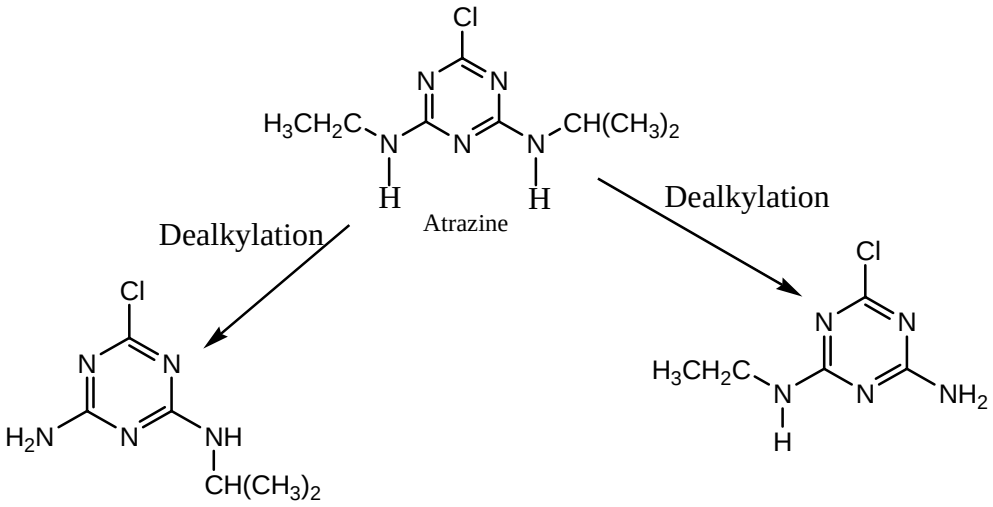


Figure 6. Degradation Product Pathway of Atrazine [36]

1.7 Methods of Extraction

During the extraction step it is usually hard to achieve high selectivity because so many unwanted compounds are co-extracted with the analytes. The first step in this process was to determine how soil samples would be taken in the field, packaged, and shipped to their respective laboratories [37-39].

Techniques for selective isolation of pesticide residues in soil matrices usually involve conventional liquid-solid extraction methods such as solvent shake and soxhlet extraction [40].The traditional or conventional extraction methods for solid samples include soxhlet extraction, solvent-shake method and sonication [41]. The modern ones are supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE) [42, 43]. The modern methods demonstrated to overcome some of the limitation of the classical methods.

Soxhlet extraction is easy to handle, however, involves large volume of solvent, which is often toxic, is needed and takes 6-24 h. The procedure is tedious and time consuming. The most popular solvents frequently utilized for soxhlet extraction are methanol; n-

hexane, acetone, diethyl ether, chloroform, acetonitrile and dichloromethane are applied [44-47].

Solvent-shake extractions require large volumes of solvents, take up to 1 h and sometimes multiple extraction is needed. The most popular solvents for this type of extraction are methanol, acetone and chloroform [47].

The extract contains a number of compounds in addition to the pesticide residues and a clean-up of the extract is needed before the final determination. Adsorption chromatography on silica, alumina and Florisil (a synthetic magnesium silicate) separate mainly according to polarities and may be quite selective depending on which eluents are applied [48,49].

1.8 Objectives of the Study

1.8.1 General Objective

The main objective of this project is to selectively extract the residues of ametryn and atrazine from soil samples of Wonji-Shoa sugar cane plantation farm.

1.8.2. Specific Objectives

The project undertaking is designed in order to meet the specific objectives out lined below: It was, there fore, planned to:

- collect soil samples from Wonji-Shoa sugar cane plantation farm.
- select the appropriate solvents for extraction techniques.
- obtain a cleaned extracts by removing non-target interferents
- determine the moisture content of the soil samples
- prepare a processed extract ready for HPLC analysis.

2. EXPERIMENTAL

2.1 Equipment and Apparatus

Mortar and Pestle were used for grinding and homogenizing the samples. Analytical balance (METTLER AT 250, Switzerland) and oven (DIGIT HEAT Abrera (Barclona, Spain) were used for weighing and drying the samples, respectively. The ground samples were sieved using 1.7 mm sieve (Standard Sieve ASTM E-11, USA). Orbital shaker (Type Ro 5, Gerhardt, Germany) was used for shaking the samples. Standard Soxhlet apparatus was used for extraction of the samples. ROTAVAPOR (BUCHI, Switzerland) was used for the evaporation of the solvent. The column used for clean-up was plugged by Glass Wool. Cellulose Extraction Timbles (ID =33 mm), ED = 35 mm), EL = 94 mm) were used for soxhlet extraction.

2.2 Chemicals and Reagents

Acetone (Analytical grade 99.5 %), Techino Pharmchem, India) was used as extracting solvent, Ammonium chloride, (98 -99.9 %) was utilized as a dispersing agent, anhydrous sodium sulfate (98 %) was the drying agent in clean up and extraction processes, Standard stock solutions were prepared by using Acetonitrile (HPLC grade, Riedel-deHaën, 99.9 %), The aluminum foil and all glass wares were rinsed by methanol (99.5 %, BDH, England), Florosil (60-100 mesh) was used for clean-up, Ametryn (99.5 %), and Atrazine (98.5%) both from GmbH, Germany were used as a primary standards, The column was pre-washed by using n-hexane (99 % pure, BDH, England) .

2.3 Soil Sampling

Soil samples were collected from wonji-shoa sugar cane plantation on May 9, 2007 (Table 4)**.** A composite soil sample (10 kg) was taken from each field. Ten holes of 25 cm depth were made randomly using a spade. Then slices of 4 cm thickness were taken along the vertical wall of the holes using spade. All increment collected were pooled on a

plastic sheet and mixed manually. To ensure further homogenization, the soil sample was divided in to six portions over the plastic sheet and then a small amount was taken from each portion to make a sub-sample of approximately 1 kg. The sub-sample of soil was kept in a polyethylene plastic bag after being wrapped in a methanol rinsed aluminium foil and then transported to the laboratory in a chilled insulating box. Before herbicide analysis, the soil samples were air-dried at room temperature, pulverized and screened through a 1.7 mm sieve and frozen at – 18 ^{o}C until extraction.

Table 4. Sample Origin

Sample	**Field No.**	**Date of Spray**	**Days after Spraying**	Spray per Hectare Gesapax Combi 500 FW
1	184	18/04/2006	13 months	3 L
2	35	17/04/2007	21 days	6 L
3	29	31/07/2006	10 months	4 L
4	12	22/03/2007	2 months	6 L
5	11	30/04/2007	10 days	7L

2.4 Extraction Procedures

2.4.1 Solvent Shake Extraction

A 5 g air-dried and sieved soil sample was mixed with 2 gm of anhydrous sodium sulfate in 250 ml Erlenmeyer flask. A soil sample of 2 gm was taken simultaneously for moisture determination. 3.5 ml of 0.2 M ammonium chloride solution was added to the flask as dispersing agent and then swirled and let to stand for 15 min. 50 ml of acetone was added as extracting solvent and shaken vigorously by hand for 1 min. It was further shaken for 4 hours using orbital shaker and kept overnight for equilibration. Using a pipette, the liquid phase was transferred into another 50 ml Erlenmeyer flask. The flask containing the

residue was rinsed with 10 ml extracting solvent and then decanted to the same 50 ml Erlenmeyer flask.

2.4.2 Soxhlet Extraction

A Soxhlet extraction was used for all soxhlet extraction with a 33 x 94 mm single layer cellulose extraction thimble (Whatman, International Ltd, 2800339, England). Extraction time was 6 hrs at a rate of 4 cycles per hour for 5.0 g of the soil sample mixed with 2 g of Na_2SO_4. 200 ml of acetone was used for the extraction. The extract was evaporated to 10-15 ml by using a rotary evaporator.

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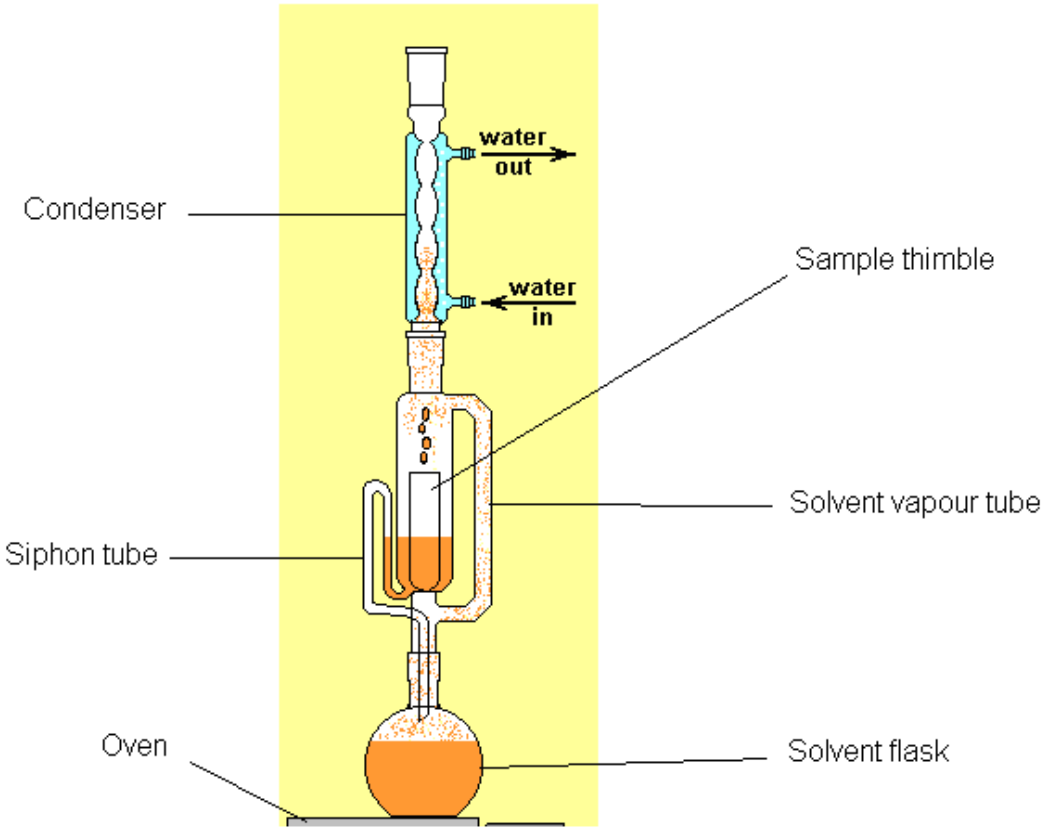

Figure 7. Soxhlet Extraction Apparatus

2.5 Extract Clean-up

The extraction column were packed with 4 g of activated Florisil (activated overnight at 200 ^{o}C) and topped with 2 g of Na_2SO_4. The column was pre-washed with 50 ml of n-hexane and just prior to exposure of sodium sulfate to air the sample in the vial was transferred onto the column. This was followed by elution with 50 ml of acetone/acetonitrile (1:1, v/v). The eluate was collected in a 250 ml round bottom flask and evaporated dryness (approximately 0.5 ml) using a rotary evaporator and then reconstituted in 1.5 ml of acetonitrile.

3. DISCUSSION

3.1 General Observation

The type of soil samples from different sampling sites was slightly different. Soil samples from sites 1, 2, and 3 were heavily muddy type but the other two samples from sites 4 and 5 are dry soil samples. But after air drying the moisture content was slightly higher in the case of dry soil samples.

The colour of the extracting sample was light yellow. After clean-up the colour of the extracting sample was more or less clear.

3.2 Moisture Content Determination

Before carrying out moisture content determination, the processed soil sample was dried overnight in an oven at a temperature of 105 ^{o}C. It should also be noted that weighing of the processed sample is performed each time when portion of the sample is taken for each type of extraction, Viz. SSE and SOX. Weight of the processed sample was taken prior to drying operation, and after drying overnight.

Percentage of the moisture content of the soil was thus determined from the result of weighing operations using the following equations.

$$\% \text{ moisture content} = \frac{\text{Initial weight- Final weight}}{\text{Initial weight}} \times 100$$

Similarly percentage of the dry weight was calculated as follows:

$$\% \text{ dry weight} = \frac{\text{Final weight}}{\text{Initial weight}} \times 100$$

Results of the weighing operations both for moisture content and dry weighing are given in Table 5 and Table 6 below:

Table 5. Percentage Amounts of Dry Weight and Moisture Content of the Soil Samples Extracted by SSE

Sample	Initial weight	Final weight	% dry weight	% moisture content
1	5.0019	4.7181	94.3	5.7
2	5.0025	4.7748	95.4	4.6
3	5.0053	4.6759	93.4	6.6
4	5.0006	4.6134	92.3	7.7
5	5.0052	4.6277	92.5	7.5

Table 6. Percentage Amounts of Dry Weight and Moisture Content of the Soil Samples Extracted by SOX

Sample	Initial weight	Final weight	% dry weight	%moisture content
1	5.0004	4.7515	95.0	5.0
2	5.0046	4.7751	95.4	4.6
3	5.0045	4.7269	94.5	5.5
4	4.9976	4.6395	92.8	7.2
5	4.9978	4.7229	94.5	5.5

The day of the extraction carried out for each sample was different. Due to this the percentage of moisture content has been varied.

4. CONCLUSIONS

Soxhlet extraction and solvent shake extraction with acetone followed by clean-up of soil extracts provided techniques are employed for extracting residues of atrazine and ametryn herbicides from the soil samples on which sugar cane was cultivated.

Following the conventional extraction approaches, stated above, extracts whose sample preparations have been nearly completed were obtained. One important observation noticed from this work is the importance of clean-up, when such sample matrices are extracted by conventional method. The clean-up step seems efficient in removing non-target compounds and other interferents.

It has not been possible to draw some general conclusions on the basis of what has been done and the results achieved. Our lab currently received a new and modern HPLC system, which will be installed in less than two weeks time. Final analysis is thus planned to follow the installation of the equipment, and this will hopefully allow us to provide some general statement for the preliminary work carried out so far.

5. REFERENCES

1. A. Perez-Ruzafa, S. Navarro, A. Barba, C. Marcos, M. A. Camara, F. Salas and J. M. Gutierrez, *Presence of Pesticides throughout Trophic Compartments of the Food Web in the Mar Menor Lagoon (SE Spain)*, Marine Pollution Bulletin, **2000**, 40(2), pp. 140-151.
2. W. Battaglin and J. Fairchild, *Potential Toxicity of Pesticides Measured in Midwestern Streams to Aquatic Organisms*, Water Science and Technology, **2002**, 45(9), pp 95-103.
3. S. A. Volz and J. J. Johnston, *Solid Phase Extraction/Gas Chromatography/ Electron Capture Detector Method for the Determination of Organochlorine Pesticides in Wildlife and Wildlife Food Sources*, J. Sep. Sci. **2002**, 25, 119-124.
4. E. Zacco, M. I. Pividori and S. Alegret, *Electrochemical Magnetoimmunosensing Strategy for the Detection of Pesticides Residues*, Anal. Chem. **2006**, 78, 1780-1788.
5. R. Zbytniewski and B. Buszewski, *Sorption of Pesticides in Soil and Compost, Polish Journal of Environment Studies*, **2002,** 11(2), 179-184.
6. A. Craven and S. Hoy, *Pesticide Persistence and Bound Residues in Soil-Regulatory Significance*, Environmental Pollution, **2005,** 133, 5-9.
7. A. Craven, *Bound Residues of Organic Compounds in the Soil: the Significance of Pesticide Persistence in Soil and Water*: a European Regulatory View, Environmental

Pollution , **2000**, 108, 15-18.

8. M. Noshadi, S. Amin and N. Maleki, *Australian Journal of Soil Research*, **2002,** 40, 643-653.
9. Y.S. Wang, J.R. Duh, Y.F. Liang and Y.L. Chen, *Dissipation of Three s-Triazine Herbicides, Atrazine, Simazine, and Ametryn, in Subtropical Soils***,** Bull. Environ. Contain. Toxicol. **1995**, 55, 351-358.
10. K.Siimes, S.Ramo, L.Welling, U.Nikunen and P.Laitinen, *Comparison of the Behaviour of Three Herbicides in a Field Experiment under Bare Soil Conditions*, Agricultural water management, **2006**, 84, 53-64.
11. J. B. Weber, K. A. Taylor and G. G. Wilkerson, *Soil and Herbicide Properties Influenced Mobility of Atrazine, Metolachlor, and Primisulfuron-Methyl in Field Lysimeters* , Agron. J. **2006**, 98, 8–18.
12. G. Jager, in: *Chemistry of Pesticides*, K.H. Buchel (Ed.), John Wiley, New York, **1983**, pp 1-9.
13. W.S. Curran, *Persistence of Herbicides in Soil*, Agronomy Facts 36, College of Agricultural Science, **1998**, 1-4.
14. A. Abdulahi, *The Status of Pesticide Registration in Ethiopia*, Pest Mgt J Eth, **1997**, 1(1-2), 57-62.
15. N. Kovacic, H. Prosen and L. Z. Kraij, *Determination of Triazines and Atraaine Metabolites in Soil by Microwave -Assisted Solvent Extraction and High-Pressure Liquid Chromatography with Phote- Diode- Array Detection*, Acta Chim. Slov. **2004**, 51, 395-407.
16. M. Gfrerer, D. Martens, B. M. Gawlik, T. Wenzl, A. Zhang, X. Quan, C. Sun, J. Chen, B. Platzer, E. Lankmayr and A. Kettrup,*Triazines in the Aquatic Systems of the Eastern Chinese Rivers Liao-He and Yangtse*, Chemosphere **2002**, 47, 455-466.
17. K. Bester, H. Huhnerfuss, U. Brockmann and H. J. Rick, *Biological Effects of Triazine Herbicides Contamination on Marine Phytoplankton,* Arch. Environ. Contam. Toxicol. **1995**, 29, 277-283.
18. **C. G.** Zambonin, F. Catucci and F. Palmisano**,** *Solid Phase Microextraction Coupled to Gas Chromatography Mass Spectrometry for the Determination of the Adsorption Coefficients of Triazines in Soil,* Analyst, **1998**, 123, 2825–2828.

19. S. Tao, L. Q. Guo, X. J. Wang, W. X. Liu, T.Z Ju, R. Dawson J. Cao, F. L. Xu and B . G. Li, *Use of Sequential ASE Extraction to Evaluate the Bioavailability of DDT and its Metabolites to Wheat Roots in Soils with Various Organic Carbon Contents*, The Science of the Total Environment, **2004**, 320, 1-9.
20. H.M. Lesan and A. Bhandari, *Evaluation of Atrazine Binding to Surface Soils,* Kansas State University, Proceeding of the **2000** Conference on Hazardous Waste Research.
21. I. D. Kovaios, C. A. Paraskeva, P. G. Koutsoukos and A. C. Payatakes, and *Adsorption of Atrazine on Soil: Model Study*, Journal of Colloid and Interface Science, **2006**, 299, 88-94.
22. S.Houot, E.Topp, A. Yassir and G. Soulas, *Dependence of accelerated degradation of Atrazine on Soil pH in French and Canadian Soils*, Soil Biology and Biochemistry **2000**, 32, 615-625.
23. G.G. Ying, R.S. Kookana and M. Mallavarpu, *Release behavior of triazine residues in stabilized contaminated soils*, Environmental Pollution ,**2005**,134, 71–77.
24. P. K. Ghosh and L. Philip, *Environmental Significance of Atrazine in Aqueous Systems and its Removal by Biological Processes: an Overview*, Global NEST Journal, **2006**, 8(2), pp 159-178.
25. R. Cherrier, A. Boivin, C. Perrin-Ganier and M. Schiavon, *Comparison of the Leaching Behaviour of two Maize Herbicides: Atrazine and Sulcotrione*, Agron. Sustain. Dev. **2005**, 25, 293-299.
26. M. Dehghani, S. Nasseri, S. Amin, K. Naddafi, M.Yunosian and N. Maleki, *Atrazine Adsorption Desorption Behavior in Darehasaluie Kavar Corn Field Soil in Fars Province of Iran*, Iran. J. Environ. Health. Sci. Eng., **2005**, 2(4), pp. 221-228.
27. A. P. Schwab, P. A. Splichal and M. K. Banks, *Adsorption of Atrazine and Alachlor to Aquifer Material and Soil*, Water, Air and Soil Pollution, **2004**, 177, 119-134.
28. E. Topp, W. M. Mulbry, H. Zhu, S. M. Nour and D. Cuppels, *Characterization of S-Triazine Herbicides Metabolism by a Nocardioides sp. Isolated from Agricultural Soils*, Applied and Environmental Microbiology, Aug. **2000**, pp. 3134-3141.
29. C. D. S. Tomlin, *The Pesticide Manual*, 14th edition The British Crop Protection Council, **2006**.

30. N Megersa, J.A. Jonsson, *Trace enrichment and sample preparation of Alkylthio-S-triazine herbicides in environment waters using a supported liquid membrane technique in combination with HPLC*, *Analyst* **1998**, 123, 225.
31. P. C. Keary and D. D. Kaufman, *Herbicides, Chemistry Degradation and Mode of Action*, Vol. 1, Marcel Dekker, New York, 1975.
32. U.S. Environmental Protection Agency. 1989. *Health Advisory Summary*: Ametryn. U.S. EPA. Washington**, DC.**
33. V. L. Lanchote, P. S. Bonato, A. L. Cerdeira, N. A. G. Santos, D.Carvalho and M.A. Gomes, *HPLC Screening and GC-MS Confirmation of Triazine Herbicides Residues in Drinking Water from Sugarcane Area in Brazil*,Water,Air, and Soil Pollution,**2000**, 118**,** 329–337.
34. P .K. Ghossh and L. Philip, *Environmental Significance of Atrazine in Aqueous Systems and its Removal by Biological Processes*: An Overview, Global Nest Journal, **2006**, 8(2), pp 159-178.
35. T. E. Stoker, D. L. Guidici, S. c. Laws and R.L. Cooper, *The Effects of Atrazine Metabolites on Puberty and Thyroid Function in the Male Wistar Rat*, Toxicological Sciences, **2002**, 67, 198-206.
36. D. S. Carter, *Determination of Atrazine and its Major Degradation Products in Soil Pore Water by Solid -Phase Extraction, Chemical Derivatization, and Gas Chromatography/ Mass Spectrometry*, Indiana polis, Indiana, U.S. Geological urvey, **1996**.
37. L. Dabrowski, H. G. Mozajska,L. Gorski, M. Biziuk, J. Namiesnik and B.Janicki, *Determination of Environmental Pollutants in Soil and Sediments-Some Aspects of Sample Clean-Up and GC analysis*, J. Sep. Sci. **2002**, 25,290-296.
38. G. N. Saxton and B. Engel, *A Survey of Soil Sample Handling Procedures of State Pesticide Regulatory Agencies***,** *Environmental Forensics*, **2005**, 6, 105–108.
39. S.P. Theocharopoulos, G. Wagner, J. Sprengart, M-E. Mohr, A. Desaules, H. Muntau, M. Christou, P. Quevauviller, *European Soil Sampling Guidelines for Soil Pollution Studies*, The Science of the Total Environment, **2001**, 264, 51-62.
40. A. Hussen, R. Westbom, N. Megersa, N. Retta, L. Mathiasson and E. Bjorklund,

Optimisation of pressurized Liquid Extraction for the Determination of p,p'-DDT and p,p'-DDE in Aged Contaminated Ethiopian Soils, Anal Bioanal Chem, **2006**,386,1525-1533.

41. A. Brachet, S. Rudaz, L. Mateus, P. Christen and J. Veuthey, *Optimisation of Accelerated Solvent Extraction of Cocain and Benzoylecgonine from Coca Leaves*, J. Sep. Sci. **2001**, 24, 865-873.
42. H.J. Vandenberg, A.A. Clifford, K.D. Bartle, J. Carroll and I.D. Newton, *Analyst*, **1999,** 24, 397-400.
43. S. Stipicevic, S. Fingler, L. Z. Kraij and V. Drevenkar, *Comparison of Gas and High Performance Liquid Chromatography with Selective Detection for Determination of triazine Herbicides and their Degradation products Extracted Ultrasonically from Soil*, J. Sep. Sci. **2003**, 26, 1237-1246.
44. M. D. Luque de Castro, *Preliminary Steps of the Analytical Process. From Sampling to detection,* Pure Appl. Chem. 2002, 74, 2293-2298.
45. L. Sun, H. K. Lee, *Microwave-Assisted Extraction Behavior of Non- Polar and Polar Pollutants in Soil with Analysis by High-Performance Liquid Chromatography*, J. Sep. Sci. **2002**, 25, 67-76.
46 . M. S. Melecchi, M. M. Martinez, F. C. Abad, P. P. Zini, I. N. Filho and E. B. Caramao, *Chemical Composition of Hibiscus tiliaceus L. Flowers: A Study of Extraction Methods*, Sep. Sci. **2002**, 25, 86-90.
47. I. Baranowska, H. Barchanska and E. Pacak, *Procedures of trophic chain samples preparation for determination of triazines by HPLC and metals by ICP-AES methods* ,Environmental Pollution **2006,** 143 , 206-211.
48. M. Akerblom, *Environmental Monitoring of Pesticides Residues, Guide lines for the SADC Region,* **1995**, Sweden.
49. Wiley & Sons, *Journal of mass spectrometry*, **1997,** 32, 897- 898.