SHORT COMMUNICATION

Determination of Selected Pesticide Residues in Diga Water Reservoir, Eastern Wollega Zone, Ethiopia

Senbeto Debelo[1], Teshome Tolcha[2], Tesfa Bedassa[1]*

[1]Department of Chemistry, Wollega University, P. O. Box 395; Nekemte, Ethiopia
[2]Kotebe Metropolitan University, 011 660 0921, Addis Ababa, Ethiopia

***Corresponding Author:** tesfabdh@gmail.com

ABSTRACT

This study aimed to assess the status of diazinon, chlorothalonil, Malathion and chloropyrifos in *Diga* water reservoir, located in *Diga* district, Eastern Wollega Zone, Ethiopia. It serves as Nekemte City municipal tap water reservoir. Salting-out assisted liquid-liquid extraction (SAALE) was used for extraction and/or preconcentration of the target analytes before their determination by gas chromatography mass spectrometry (GC-MS). The method showed acceptable linearity (R^2) in the range of 0.996 to 0.999. The limit of detection (LOD) of the method ranged from 1.38 to 4.92 µg $L^{-1}$and limit of quantification (LOQ) ranged from 4.60 to 16.40 µg L^{-1}. The relative recovery ranged from 80.34 % to 100.32%. Analysis of the collected real samples indicates that the water samples were not polluted with residues of diazinon, chlorothalonil, Malathion and chlorpyrifos or contain below the LOD of the method.

Keywords: Gas chromatography-mass spectrometry; Pesticide residues; Reservoir water; Salt assisted liquid-liquid extraction.

INTRODUCTION

The use of chemical pesticides to kill insects that transmit infectious micro-organisms and increase crop production by controlling pest infestation in agriculture is very common all over the world (Dujakovic et al., 2010; Grube et al. 2011; Skevas et al. 2013; Jansen and Dubois 2014). Among the applied pesticides, less than 0.1% reaches the targeted pests, while the remaining 99.9% remain as residues; these are potentially able to contribute to environmental pollution. Pesticide residues do not remain on the target area; they are transported by surface run-offs during the rainy season to water bodies. Thus, large fractions of pesticide residues in agricultural areas are taken into streams, rivers, and lakes. Some amount of these leach to the groundwater systems or volatilize to the atmosphere. The run-off occurring within a few days of a pesticide application typically removes about 1% of the applied amount of pesticides to soil (Sabik et al., 2000; Hung and Thiemann, 2006; Pinheiro and Andrade, 2009; Dasgupta et al., 2011). Consequently, the pesticide residues have been detected in various types of water circulating in the ecosystem; these harmful chemicals could, therefore, be exposed, creating a potential risk to life and health of all living organisms in the environment (Tankiewicz et al., 2010; Tankiewicz et al., 2011). Residues of persistent pesticides could be retained in the grown crop; they can directly or indirectly pollute food and food products and biological systems (Gilliom, 2007). Moreover, pesticide residues may also be capable of bioaccumulation. Bioaccumulation of pesticide residues depends on several factors, including lipophilicity, hydrophobicity, and chemical and biological degradation rates. Lipophilic pesticides with a low rate of degradation accumulate in the biological tissues and cause a subsequent magnification of concentrations in the organisms (Tankiewicz et al., 2011; Pandey, 2010).

In Ethiopia, herbicides and insecticides are used to increase crop production and for public health programs. Organochlorine pesticides (OCPs) and organophosphorus pesticides (OPPs) are among the commonly distributed pesticides in rural areas of the country (Dirbaba et al., 2018; Adinew et al., 2017). OCPs and degradation product of some OPPs are categorized as a group of persistent pollutants, at a global level. Most of these compounds have been prohibited from use due to their toxic effects (Munch, 2009; Dessie and Kinlund, 2008). However, the use of OCPs and OPPs for agriculture and health purposes in developing countries has led to widespread pollution of the environment. Owing to their properties, including low volatility, chemical stability, environmental resistance, lipophilicity and slow metabolic degradation, bioaccumulation of these pesticides has occurred in birds and mammals and the food chain. (Wiktelius and Edwards, 2009). Hence, among the studies required to bring about a favorable and healthy environment, monitoring of environmental water quality has to include the assessment of these compounds.

Salting-out assisted liquid-liquid extraction (SALLE) provides some attractive virtues such as simplicity, fast phase separation, good compatibility with subsequent gas chromatography mass spectrometry (GC/MS) determinations. Acetonitrile is a promising extracting solvent in SALLE since it is miscible with water in all proportions; its polarity is favorable for the extraction of a wide range of compounds. Furthermore, acetonitrile is less harmful than the solvents used in conventional liquid-liquid extraction; this makes it more favorable within a green chemistry context (Cai et al., 2007; Razmara et al., 2011). Gure et al. (2014) applied SALLE in combination with capillary High-Performance Liquid Chromatography (cHPLC) for the determination of sulfonylurea herbicides in environmental water. Tolcha and Megersa (2018) used high-density solvent-based dispersive liquid-liquid microextraction technique (DLLME) for the extraction of selected pesticide residues in water and sugarcane juice samples. However, there is no report on the status of pesticide residues in a tap water reservoir of Nekemte town constructed in Diga district. Hence, this study aimed to assess the level of selected pesticide residues (diazinon, chlorothalonil, malathion & chloropyrifos) in Nekemte City tap water reservoir, which was constructed in Diga district, East Wollega zone, Ethiopia.

MATERIALS AND METHODS

Description of the sampling site

This study was conducted at Nekemte City municipality water supply reservoir located in Diga district, East Wollega Zone, Oromia Regional State, Ethiopia (Fig. 1). Diga district is about 12 km far from Nekemte City and 345 km from Addis Ababa, Ethiopia. The community in this area had been using organochlorine pesticides for treatment of insects that used to infect cattle; some organophosphorus pesticides have also been in use for a similar purpose. The residues of these pesticides could be added to soil and water in the area; hence a study is required to assess the status of the water supply that is currently used as a major source of Nekemte community.

Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade while the solvents were of chromatographic grade. Among the chemical used were 2-propanol (Carlo Erba Reactifs-SDS, Chaussée du vekin, Val de Revil, France), acetonitrile (Ashland chemical; S. Giuliano MI, Italy), and Ammonium sulphate (NH_4SO_4) (Fluka; Buchs, Switzerland). Ultrapure water used in this study was purified with double distiller A8000 Aquatron water (Bibby Scientific Ltd, Staffordshire, United Kingdom) and deionizer (Thermo Scientific Barnstead E-Pure™, Thermo Fisher Scientific Inc., Italy). Analytical standards of diazinon (99.9%), chlorothalonil (99.3%) and chlorpyrifos (99.7%) were purchased from Sigma Aldrich (St. Louis, MO, USA). Malathion (99.3%) was supplied by Dr. Ehrenstorfer (Augusburg, Germany). Standard stock solutions, 1000 mg L^{-1}, of each targeted analyte (diazinon, Malathion, chlorothalonil and chlorpyrifos) were prepared in 2- propanol. The working solution contains 2 mg L^{-1} of each standard pesticide was obtained by mixing each standard diluting with de-ionized water.

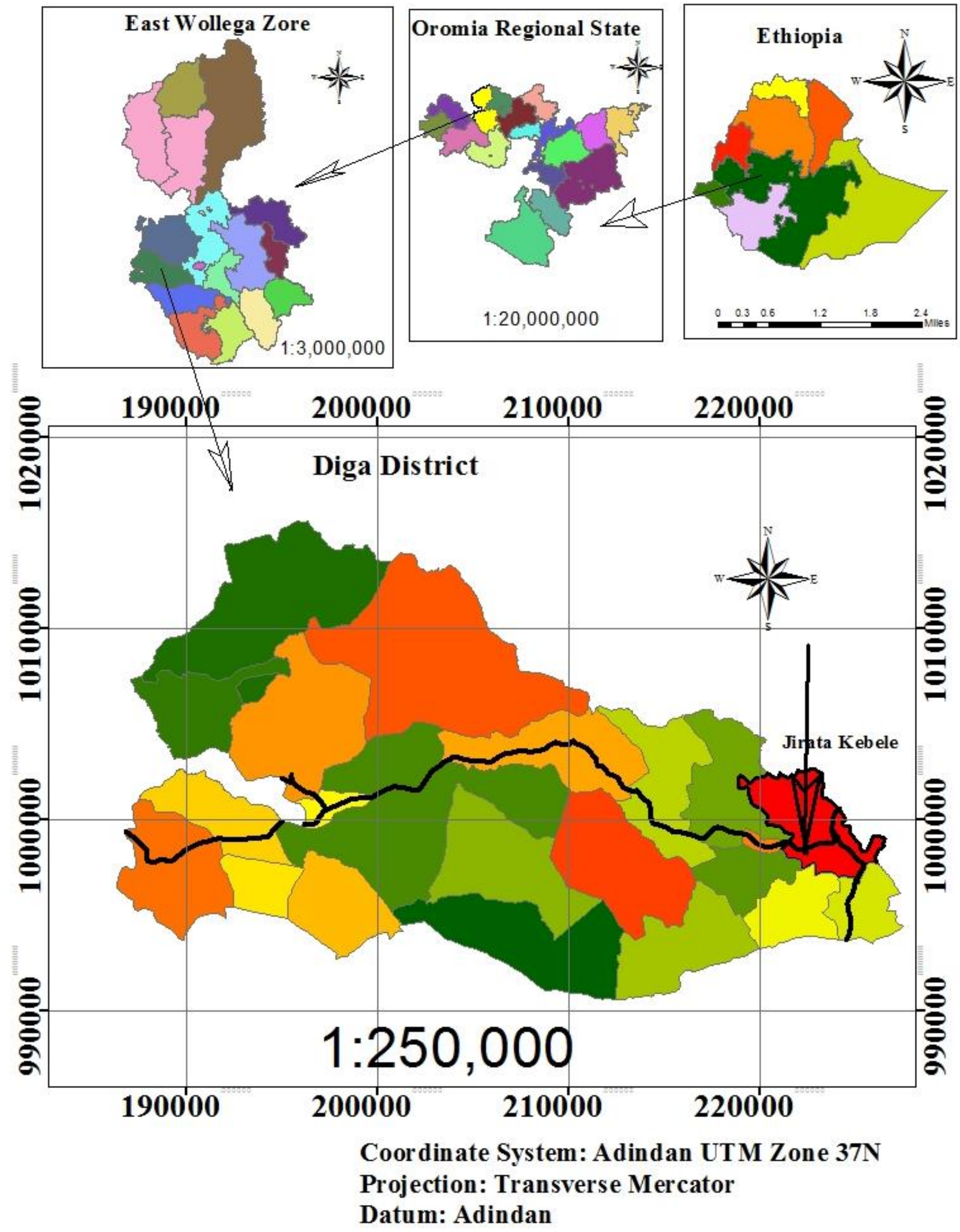

Fig. 1. The geographical location of the reservoir.

Apparatus and Instruments

The GC-Ms instrument used was Agilent Technologies, 7820A gas chromatography system equipped with Agilent Technologies, 5977E inert mass spectrometry (MS) detector. The GC column was Hp-5ms ultra inert capillary column (25 m x 250 µm and 0.25 µm i.d.). Amber glasses were used for sampling. The apparatus and equipment used during SALLE included separating funnel, centrifuge, 15 mL falcon centrifuge tube, 5 mL and 3 mL syringe, and 2 mL autosampler vials.

Sampling Techniques

Grab sampling method, in which a single increment is taken at a specific site, was used to get representative samples during all sampling periods (Yusef et al., 2019). Before sampling, five amber glasses were washed with detergent, rinsed with distilled water and pure acetone (99.9%). The glass bottles were then allowed to dry in an oven and carefully labeled for sampling. Four different sampling locations of the reservoir were purposely selected. These different sites were identified as Northern, Southern, Eastern and Western parts of the reservoir and then, specific sampling site of each direction was chosen randomly.

Samples were collected at 1cm depth from each site. Then, a 1L composite sample was formed by mixing the collected samples at the sampling site. Similarly, 1L sample was also taken from treatment station. Glass bottles containing the water samples were sealed and transported to Analytical Research Laboratory of Addis Ababa University, Ethiopia, for analysis.

The collected samples were then kept in the laboratory refrigerator at 4°C until used for analysis. Before SALLE, the water samples were filtered with Whatman No. 42 filter paper to remove suspended impurities and particulate matters.

SALLE procedure

For the SALLE (Wen, et al., 2013), 4 mL water sample was transferred into a 15 mL falcon centrifuge tube with conical bottom and then, 1 mL of acetonitrile was added, followed by gentle shaking with a vortex mixer for about 30 seconds. Then, 1.6 g $(NH_4)_2SO_4$ was added to the mixture and then, further vortexed for 3 min. At this stage, the content was separated into two phases and the analyte was extracted into the upper organic phase (acetonitrile phase). To facilitate phase separation of the upper organic phase, the mixture was centrifuged for 5 min at 9000 rpm. The supernatant was then withdrawn using a 1mL syringe with a sharp needle tip and transferred to a 2 mL autosampler vial and injected into GC-MS.

Methods of analysis

After SALLE, the target analytes were separated and quantitatively analyzed by GC-MS using helium gas (99.999%) mobile phase at a flow rate of 1 mL min^{-1}. The GC oven temperature program was set at 130 ^{o}C for 0 min; increased at 25 ^{o}C min^{-1} to 185 ^{o}C min^{-1} and then held constant for 1 min; then increased at 9 ^{o}C min^{-1} to 200^{o}C for 1 min and 10^{o}C min^{-1} to 290^{o}C and then held for 1 min. GC oven and injection port temperature were maintained at 290 ^{o}C and 250 ^{o}C, respectively. Injections were made in split-less mode and the mass detector was used in the full scan mode, over the range of m/z 50–550. Selective ion monitoring (SIM) mode was used for the determination of the analytes. For quantitative determinations, calibration curves were constructed at five concentration levels ranging from 8 µg L^{-1} - 128 µg L^{-1}.

Method validation

Limit of detection and quantification

Limit of detections (LOD) and quantifications (LOQ) was determined by signal-to-noise (S/N) ratio method at a concentration that gives 3 and 10 times S/N, respectively (Ribani et al., 2007; Snyder, 2010).

Precision studies

The precision of the method was examined in terms of repeatability, by analyzing replicate samples (n=3) and as relative standard deviations (RSD).

Recovery studies

Recovery studies were carried out, in terms of per cent relative recovery (%RR), by spiking of de-ionized water and reservoir water sample with a known concentration of the analytes i.e. 32.0 µg L^{-1} of each pesticide. The spiked de-ionized water and real water samples were then extracted with SALLE, and then, analyzed by GC-MS. The peak areas of the analytes from the reservoir samples were compared with the peak areas obtained from de-ionized water. The %RR was calculated using the following equation:

$$\%RR = \left(\frac{Peak\ area_{extracted\ spiked\ sample} - Peak\ area_{extracted\ unspiked\ sample}}{Peak\ area_{extracted\ de-ionized\ water}}\right) x\ 100$$

RESULTS AND DISCUSSION

The enrichment factor of the SAALE

This is a measure of pre-concentration of the analytes as they are transferred from the aqueous sample to the organic phase. In this work, extraction of the analytes from the aqueous sample to the organic phase was carried out for compatibility of the solvent with GC. In this work, the enrichment factor was calculated as the ratio of the concentration of the analytes in the extracting solvent (acetonitrile) to the concentration of the analytes in the aqueous phase (after spiking). The concentration of the analytes in acetonitrile is calculated from the peak areas of the analytes. The enrichment factor of the extraction method is given in Table 1. It can be seen that enrichment was not achieved for diazinon by SALLE method. This could be due to greater solubility of the analyte in water than in acetonitrile. The highest enrichment factor was observed for Malathion.

Table 1. Enrichment factors of analytes by the SALLE method.

Analytes	The concentration of analytes (µg/L)		Enrichment factor
	Before extraction	After extraction	
Diazinon	32	30	0.94
Chlorothalonil	32	36	1.13
Malathion	32	143	4.50
Chloropyros	32	48	1.50

Chromatographic identification

The presence of the target analytes in the reservoir water sample was identified by comparison of their retention time to the retention times with the chromatogram of the standard solution. Furthermore, the MS spectrums of the analytes of interest were used for confirmation. Fig. 2 shows GC/MS chromatogram of the target pesticides obtained after extracting using SALLE from spiked de-ionized water. Chromatographic separation was satisfactory for the target analytes in a short time.

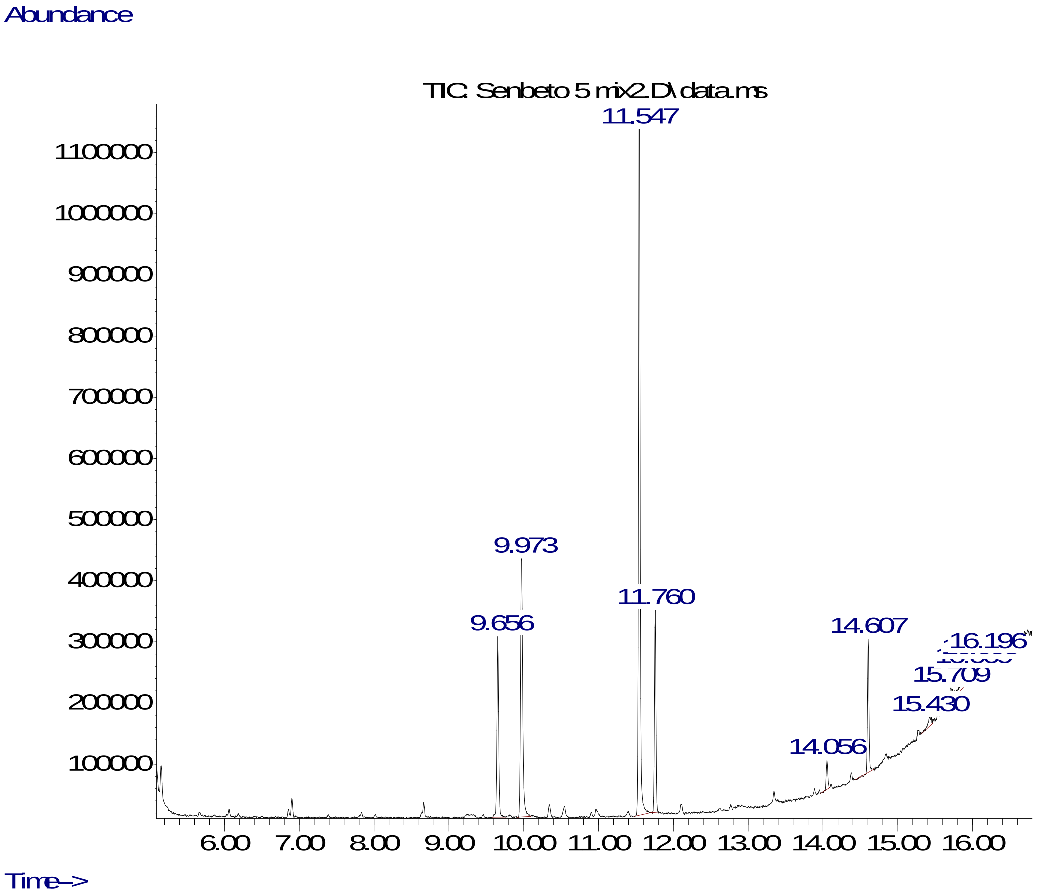

Fig. 2. A chromatogram showing extracted pesticide standards from de-ionized water. (Diazinon, RT = 9.656 min; Chlorthalonil, RT = 9.973 min; Malathion, RT = 11.547 min and Chloropyrifos, RT = 11.760 min)

Analytical performance characteristics

The analytical performance parameters including linearity, LOD, LOQ, %RR, and precision in terms of RSD are as presented in Table 2.

Table 2. Analytical performance of SALLE- GC/MS for analysis of the target pesticides from water

Analyte	Quantification ion (m/z)	Linearity (R^2)	LOD (µg L^{-1})	LOQ (µg L^{-1})	%RR	RSD (n=3)
Diazinon	152.1, 137.1, 124.1	0.999	4.92	16.40	80.34	3.98
Chlorthalonil	267.9, 265.9, 263.9	0.996	4.44	14.80	94.14	4.77
Malathion	178.1, 174.1, 158.1	0.997	1.38	4.60	86.09	6.50
Chlorpyrifos	198.9, 196.9, 179.1	0.998	4.74	15.80	100.32	2.36

The linearity was assessed by the coefficient of determination (R^2) that was in the range of 0.996 to 0.999. The LOD were ranged from 4.44 to 4.92 µg L^{-1} and LOQ were ranged from 14.80 to 16.40 µg L^{-1}. %RR were varied from 80.34 % to 100.32% for the reservoir water. Furthermore, the values of other parameters including linearity, recovery and precisions of the method were consistent with other reported values for the analysis of pesticides in water (Hanan et al., 2016; Menezes et al., 2016; Abdullah et al., 2017).

Analysis of real water samples

The studied analytes were not detected in both water samples collected from the reservoir and treatment station (Figure 3). This may be due to fact that these pesticides are either not available in the water or their concentration is lower than the LOD of the method. On the other hand, these pesticides are known to have a short lifetime in the aqueous system (Hodgson, 2004) and may account for their absence in the water sample under the current detection methods used.

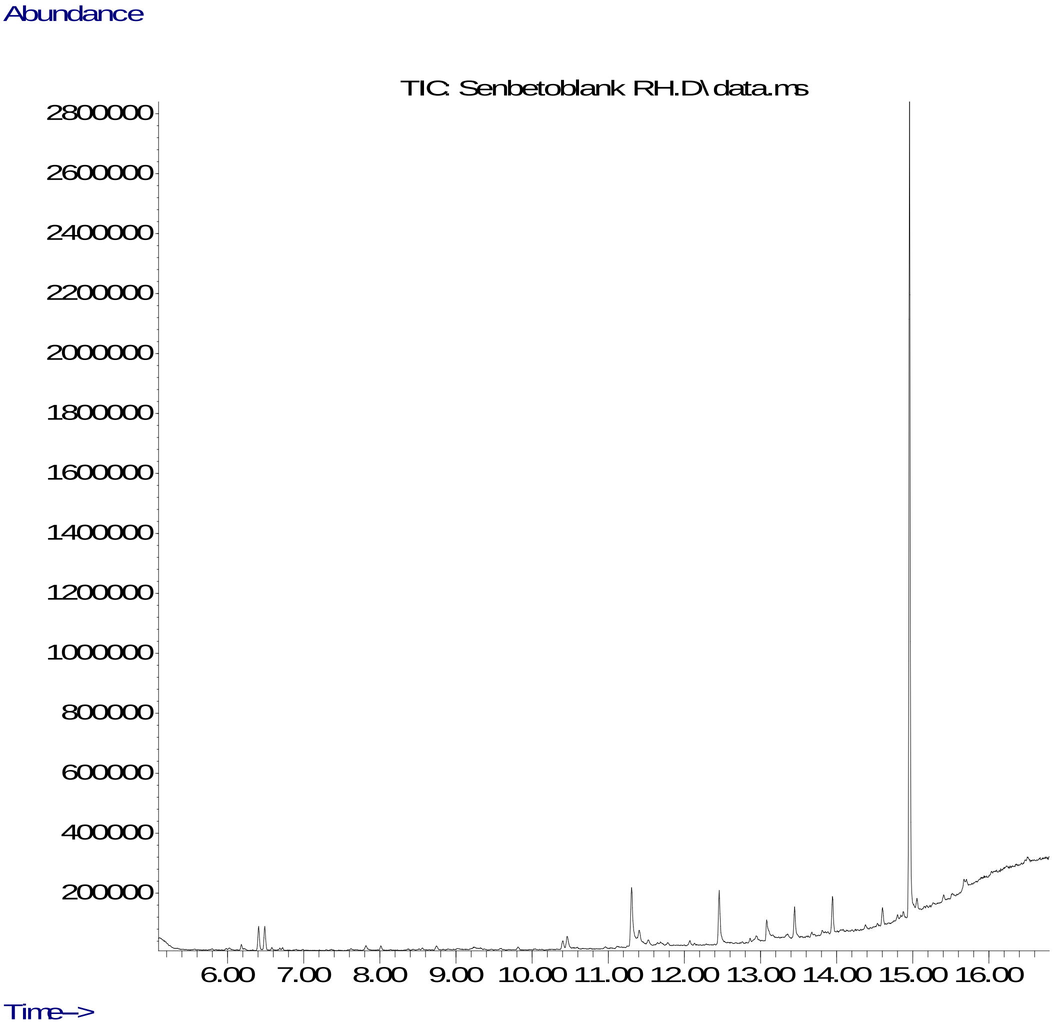

Figure 3. Chromatogram of reservoir water (RW) sample.

CONCLUSION

Water samples collected from Nekemte City municipality water reservoir at Diga district were analyzed by SALLE-GC/MS. The water samples were assessed for four pesticide residues, namely diazinon, chlorothalonil, Malathion and chloropyrifos. None of these pesticides was detected in the current analysis. It is recommended that further analysis better made on these municipal water reservoirs by more refined sampling technique both in locations and seasonally varied period of sample collection.

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