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SAMPLE PREPARATION METHODS FOR QUANTITATIVE EXTRACTION OF TRIAZINE AND ITS METABOLITES IN SEDIMENT SAMPLES OF AWASSA AND ZIWAY LAKES

MSc. GRADUATE PROJECT

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LIST OF ABBREVIATIONS

Abbreviations	Descriptions
2, 4-D	2,4-dichlorophenoxy acetic acid
%	percentage
^{o}C	Degree centigrade
mL	milliliters
g	gram
mg	milligram
L	Liter
min	minute
DEA	Deethylatrazine
DIA	deisopropylatrazine
DDA	Deethyldeisopropylatrazine
HA	Hydroxylatrazine
HPLC	high performance liquid chromatography
TLC	Thin layer chromatography
MEKC	micellar electroknetic chromatography
CE	capillary electrophoresis
cGC	capillary gas chromatography
SPE	solid phase extraction
SPME	Solid phase micro extraction
MASE	Microwave assisted solvent extraction
ASE	Accelerated solvent extraction
Kg/ha/yr	kilogram per hectare per year
ha	Hectare
E- Flask	Erlenmeyer flask
v/v	volume by volume
μ	Micro

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Abstract

A total of 21 sediment samples were collected from Awasa and Ziway Lakes for triazine herbicide residues and their metabolites analysis. The samples were air dried and extracted following standard procedures. All of the organic extracts were yellow colored and also some particulates were present. For this reason and for the possible presence of macro-organic compounds a florisil clean up was carried out. The intense yellow color of the extracts was significantly reduced but it was not totally removed, therefore, further clean up by activated charcoal is required. Moreover, the moisture contents of the sediment samples were also determined prior to the analyte extraction. It was found that the moisture contents determined were around 1 %, except fro one sample collected from Ziway whose value was around 3.8 %. The relatively high value obtained here could be due to muddy nature of the sample, which could also be seen visually. The extracts were stored at $4^{o}C$ in a refrigerator for future HPLC analysis.

1. INTRODUCTION

1.1. Pesticides

Modern agricultural practices often include the extensive use of a wide range of pesticides for increased crop production as well as for greater yield by controlling pests [1, 2]. The use of pesticides, therefore, continues to exist as world population and the demand for food production continues to grow. In 1995, conventional pesticide use in USA amounted about 1.22 billion pounds, which was one fifth of the world's use of such chemical [2].

In spite of the undeniable advantages that pesticides have brought to modern agricultural economy for controlling pests, they can generate a series of problems for untargeted organisms, if the necessary precautions are not taken during applications and storages [3]. Large fractions of the pesticides used in agricultural settings end up moving with surface run–off into streams, rivers and lakes, leaching to the ground water systems, or volatilize to the atmosphere [4]. As a result, residues of such compounds can be the main source of environmental pollution [5], they may be found in the soil in which the crop was grown, may also appear in the atmosphere, in run-off water following heavy rain, irrigation, in ground water or in surface water [6] and consequently, they can directly or indirectly pollute food and food products and biological systems.

In general, agricultural chemicals, while often benefiting farming productivity, can have determinable environmental effects when applied improperly [7]. Therefore, pesticides and their potentially undesirable effects on the environment, aquatic organisms and human health has been one of the major concerns of recent research [1]. At present, pesticides of 32 billion dollar have been marketed in the world. Among all the pesticides, herbicides production and its use are more than any other pesticides in the world market [2].

1.2. Use of Herbicides

Weed is any plant, either a wild or cultivated variety, which is undesired in that particular place. In agricultural and horticulture weeds are any plants other than specific crop being grown. Weed management or regulation of weed emergence and growth is a very important process for crop cultivation to prevent yield reduction by competition with weeds [8]. Farmers have been used methods such as hand cultivation and crop rotation to control weeds and other pests, to improve crop yields since beginning of recorded history [4].

Recently, chemical weed controls with effective and highly active herbicides have become very useful and convenient means [8]. Herbicides are substances or cultured biological organisms used to kill or suppress the growth of unwanted plants and vegetations selectively or non-selectively [9]. Thus it has brought stable crop production and is labor saving, they protect crops from undue competition from weeds and enhance the nutritional quality of food. They are generally used as pre- and post-emergence for the control of weeds in agricultural crops [10].

The development of weed control by chemical means is closely connected with the use of inorganic chemicals [11] up to World War II. A variety of inorganic acid and salts (e.g. iron (III) sulfate, sulfuric acid, and copper (II) sulfate) were applied to control weeds [11].

Weed control by chemical means has under gone rapid expansion since the introduction of selective organic herbicides after the end of World War II. Since 1945 when use of the first herbicides 2, 4-D was started, a great progress in agriculture especially in highly developed countries was observed [12]. Therefore starting with the introduction of 2, 4-D, organic herbicides made a decisive breakthrough into selective chemical weed control [11]. It kills many broad leaf plants while leaving grasses largely unaffected.

Triazine families of herbicides, introduced in 1950s, are one of the largest classes of agrochemicals produced and they are among the most commonly used herbicides. A

report indicated that, about 30% of herbicides produced are triazines [13]. The herbicidal properties of the s-triazines were discovered in 1952 by a research group of J. R. Geigy in Basel, Switzerland. The first patent applications were made in 1954 covering 2-chloro-4, 6- bis (alkyl-amino)-s- triazines and their influence on growth of plants [14]. They are being used as selective pre-and post- emergence for the control of leafy and grassy weeds in many agricultural crops like corn, soybeans, wheat, maize, sugar cane and barley [15, 16] including green vegetables [13]. They are selective pre-emergent broadleaf (dicot) herbicides that produce a reversible inhibition of photosynthesis while causing little or no injury to corn, sorghum, or sugarcane [17]. The selective action of these compounds and their unique herbicidal properties compared with existing classes of herbicides were first reported in 1955 [14]

Of all the triazines, atrazine (2-chloro-4-ethylamino-6- isopropylamino-1, 3, 5-triazine) is the most prominent derivative. It is applied to soils as selective pre- and post - emergence [18] for weed control of broad leaf and grassy weeds in maize and in other crops [19], xand as a weed killer (total herbicide) on roads and rail way tracks [18]. At present it has been used in more than 70 countries world wide to control variety of weeds, primarily in the production of corn as well as in sorghum and sugarcane [20-22].

1.3. Physico-chemical Properties of Triazine Herbicides

Most of the traizine herbicides have a structure of N-substituted benzene ring with R-groups attached to the carbon atoms. They are the derivatives of symmetrical (*s*)-triazines [23]. With few exceptions the s-triazines used as selective or general herbicides are substituted diamino -s-triazines which have commonly chlorine (the common name ending with –azine), methoxy (ending with-tone), methylthio (ending with- etryn) groups attached to the third ring carbon atom [14, 23]. Different substitutions at the 2- , 4-, and 6-positions on the triazine ring have produced a wide range of physical, chemical, and biological properties of the compounds [24]. The chemical properties of s-triazine derivative compounds can be determined primarily by the constituents in position -2 such as chlorine, methoxy and methylthio groups [14, 23]. Various alkylamino groups usually substitute positions 4 and 6 [23].

The remarkable stability of s-traizine derivatives can be explained by the electronic configuration of the heterocyclic ring which resembles that of benzene to a certain extent. Both ring systems are stabilized by delocalization of their π-electrons which spread over all six ring atoms. However, essential differences exist in electronic configuration between s-triazine and benzene as a consequence of the greater electronegativity of the nitrogen atom compared to that of the carbon atoms. Thus the π-electrons in the s-triazine ring are localized in the vicinity of nitrogen atoms rather than being evenly distributed over the whole ring. The delocalization effect in combination with inductive and mesomeric effects exerted by the substituents at C-2, C-4 and C-6 greatly influences the chemical behavior and physical properties of the s-triazine derivatives [14].

Triazines are relatively polar and have a log K_{ow} between 1.2-3.7. Dialkyl amino –s-triazines have low solublities in water, the 2-chloro s-triazine being less soluble than the 2-methylthio and 2-methoxy analogs. Depending on the moieties in the R-positions the aqueous solubility of triazines range between 5-750 mg/L [14, 23,], practically independent of the pH of the solution. However a pronounced increase in solubility is observed at pH values where strong protonation occurs, e.g. between pH 5.0 and 3.0 or lower for 2-methoxy and 2-methylthio-s-triazines and at pH 2.0 or lower for 2-chloro s-triazine [14].

Structural modifications of the substance at either 2 or 4 and 6 positions of dialkylamino-s-trriazines significantly affect solubility at all pH levels. Generally increasing solubility is associated with increasing electro-donating capacity of the substituents at C-2 and decreasing size and branching of N- alkyl groups in the 4 and 6 positions. Differences in moleculer symmetry, and therefore in molecular polarity can account for the higher solubilities of 4, 6-asymmetrically substituted 2-chloro-s-triazines such as atrazine as compared to that of symmetrically substituted compounds such as simazine and propazine [14].

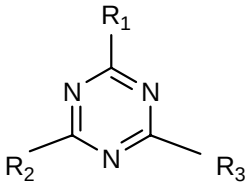


Fig.1. General Structure of *s*-triazine

Table 1: Substituents and physical constants of the s-triazine herbicides

common name	Substituents			Solubi - lity	melting point,°C	pK_a, value	Absorption maxima,nm		log K_{ow}
	R_1	R_2	R_3				λ_1	λ_2	
Simazine	Cl	NHC_2H_5	NHC_2H_5	5	225-227	1.65	222	263	1.21
Atratone	OCH_3	NHC_2H_5	$NHCH(CH_3)_2$	1650	-	4.20	217	-	1.08
Atrazine	Cl	NHC_2H_5	$NHCH(CH_3)_2$	33	175-177	1.68	222	263	2.7
Prometrone	OCH_3	$NHCH(CH_3)_2$	$NHCH(CH_3)_2$	750	91-92	4.20	219	-	2.84
Propazine	Cl	$NHCH(CH_3)_2$	$NHCH(CH_3)_2$	8.6	212-214	1.85	221	268	2.97
Terbuthylazine	Cl	NHC_2H_5	$NHC(CH_3)_2$	8.5	177-179	1.94	223	263	1.62
Prometryn	SCH_3	$NHCH(CH_3)_2$	$NHCH(CH_3)_2$	48	118-120	4.10	223	-	3.34
Terbutryn	SCH_3	NHC_2H_5	$NHC(CH_3)_2$	58	104-105	4.30	223	-	3.74

Solubility in water (mg/L) at 20-25-°C **[14, 23]**

It is evident from the pK_a data listed in table 1 that substituents at the two positions of 4, 6- bis (alkylamino) *s*-triazines most significantly affect the basicity which increases in the order of chloro-, methylthio-, methoxy s-triazine. Thus basicity increases with increasing the electron donating power of the substituents [14].

The mobility and transportation of herbicides in the environment depends on the physical and chemical properties of the compounds such as water solubility, vapor pressure, volatility, soil retention, acidity and K_{ow}. Other factors include soil texture, organic matter content of the soil, soil pH, and intensity of use and method of application, temperature and duration of rainfall, topography and moisture [2, 19].

All triazine herbicides are considered some what persistent in water and mobile in soil. The physio-chemical properties of triazines make them especially susceptible to leaching into ground water and runoff from the site of application to surface waters [2] especially during heavy rains. Because of their high water solubility, triazines have a large potential for movement into a solution and only a moderate potential for soil sorption. These properties have resulted in the contamination of surface and ground waters [17].

1.3.1. Properties of Atrazine

Atrazine is the part of triazine family of chemical compounds with six member ring containing three carbon and three nitrogen atoms. In addition to the aromatic carbon /nitrogen ring of atrazine it also contains one chlorine, ethylamine and isopropyl amine groups attached to the ring. The chemical name of atrazine is 2-chloro-4-ethylamine-6-isopropylamino-s-triazine and the chemical structure is shown below:

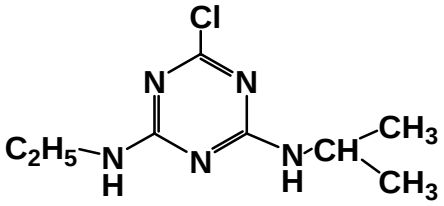


Fig .2. Structure of Atrazine

It is a colorless powder [25.] which is almost non-volatile [2] and relatively stable in neutral, weakly acidic and weakly alkaline media. Rapidly hydrolyzed to hydroxy derivatives in strong acids and alkalis, and at 70 ^{o}C in neutral media [25]

The solubility of atrzine in water is 33 mg/L (pH 7) at a temperature of 22^{o}C where as, solubility in methanol, ethylacetate, dichloromethane, chloroform, acetone, ethanol, toluene, n-hexane and n-octanol are 18, 24, 28, 52, 31, 15, 4, 0.11 and 8.7, respectively (all in g/ L at 25 ^{o}C) [2, 25].

Due to its excessive usage, high persistence and mobility, atrazine along with its metabolites are transported to surface and subsurface water bodies and been found in ground water, rivers, high mountain lakes, drinking water supplies, rain water and even in fog [2].

Degradability of agrochemical compounds in the air, soil and water is the most important factor determining their fates in the environment, and possibility of occurrence of the adverse effects on human organism [26].

Pesticides used in agriculture are mainly adsorbed and degraded in topsoil [19]. Degradation and transport rates of triazine herbicides in the environment are known to be site-specific and are dependent on soil properties such as organic carbon content, temperature, pH, and nutrient status, moisture, microbial activities and other environmental conditions [8, 27]. Increasing temperature and moisture, low pH and high organic matter content favored the hydrolysis of chloro triazines. However with the exception of low pH, these are conditions that are also favorable for microbial growth [27, 28].

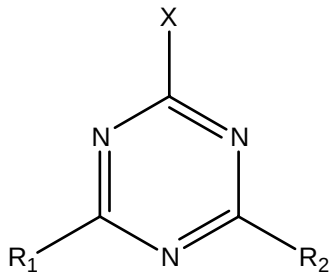
Atrazine degrades through a combination of physical, chemical and biological processes [29]. It is believed that hydrolysis and dealkylation share the primary steps of chloro s-traizine herbicide degradation. Most of the breakdown usually occurs by chemical hydrolysis and degradation by soil microorganisms. Hydrolysis of atrazine produces hydroxyatrazine substituting chlorine atom from its 2-position. It is widely accepted that HA formed by dechlorination reaction which is due to chemical process while dealkylation reactions are biologically mediated (microbial degradation). Dealkylation is the primary mechanism for the dissipation of atrazine from the environment which results the formation of DIA, DEA and DDA. The degradative path way of atrazine to its dealkylated form is proceeding through the removal of the ethyl side chain generally preceding the removal of the isopropyl group [2, 30]. The three major degradation products of atrazine at all conditions are deethylatrazine (DEA) deisopropyl atrazine (DIA) and hydroxylatrazine (HA) [29, 31]. DEA and DIA form primarily through biological process in the soil. DEA accounts for small part of atrazine degradates in soil. The presence of DEA in soil is primarily due to a large number of microbes, high organic content, and relatively warm soil temperature. Although DEA accounts for only a small part of atrazine degradates; it is significant compound in surface water because of its selective removal from soil. The chemical hydrolysis of atrazine to hydroxyatrazine (HA)

also occurs in surface soil [32, 33]. HA binds to soils with high organic matter content and low pH. Since HA binds more tightly to the soil and may be transported to streams by soil erosion due to rainfall run -off [29]. In addition, Photodgradation of atrazine on soil surface produces DEA and diaminochlorotriazine [32]

The degradation of atrazine in aquatic environment may proceed at rates that are considerably lower than those in surface soil due to lower microbial activity, nutrient availability, soil type and other environmental conditions such as temperature, aeration and moisture content [33]. However, the microbial degradation products of atarzine are the significant compound in surface and ground water because of their high relative mobility [32].

Atrazine tends to persist in both ground water and fresh water bodies. Its persistence in water varies from 4 to 57 weeks [2], but in lakes which are characterized by cold water temperatures, low productivity, high pH, low nitrate and low dissolved organic carbon, atrazine can persist for years. Atrazine in the aquatic system degrades more rapidly in the presence of emergent vegetation than in open water [30]. In some cases, no degradation of atrzine occurs may be due to its moderate tendency to bind to sediments [34]. When atrazine is degraded in aquatic system its main degradation products are dealkylated chloro metabolites, predominantly, diethylatrazine (DEA) and deisopropyl–atrazine (DIA)[17]. The principal degradation routes of atrazine are shown in Fig.4 and its degradation products are shown in Fig.3.

Chemical degradation of the s-triazine herbicides, primarily to their hydroxy analogue occurs either at high or low pH [2, 14]. Alkaline hydrolysis likely involves direct nucleophilic displacement of Cl^- from 2– position of atrazine by OH^-; where as acid hydrolysis may result from protonation of a ring or chian nitrogen atom followed by cleavage of C-Cl bond by water. It is also reported that the chemical degradation of atrazine in soil was due to the removal of chlorine by hydrolysis, catalyzed by clay and organic matter which generate hydroxyatrazine. It is rapid in acidic or basic environments, but slower at neutral pH [2].



X (2), R-1(4) and R-2(6) are the substituted groups in s-triazine in 2, 4 and 6 positions

Compound name	**X**	**R-1**	**R-2**
Atrazine	Cl	C_2H_5NH	CH_3CHCH_3NH
Hydroxylatrazine	OH	C_2H_5NH	CH_3CHCH_3NH
Deethylatrazine	Cl	NH_2	CH_3CHCH_3NH
Deisopropylatrtazine	Cl	C_2H_5NH	NH_2
Deethyldeisopropylatrazine	Cl	NH_2	NH_2
Deethylhydroxyatrazine	OH	NH_2	CH_3CHCH_3NH
Deisoprophylhydroxyatrazine	OH	C_2H_5NH	NH_2

Fig. 3. Structure of Atrazine and Its Transformed Products **[2].**

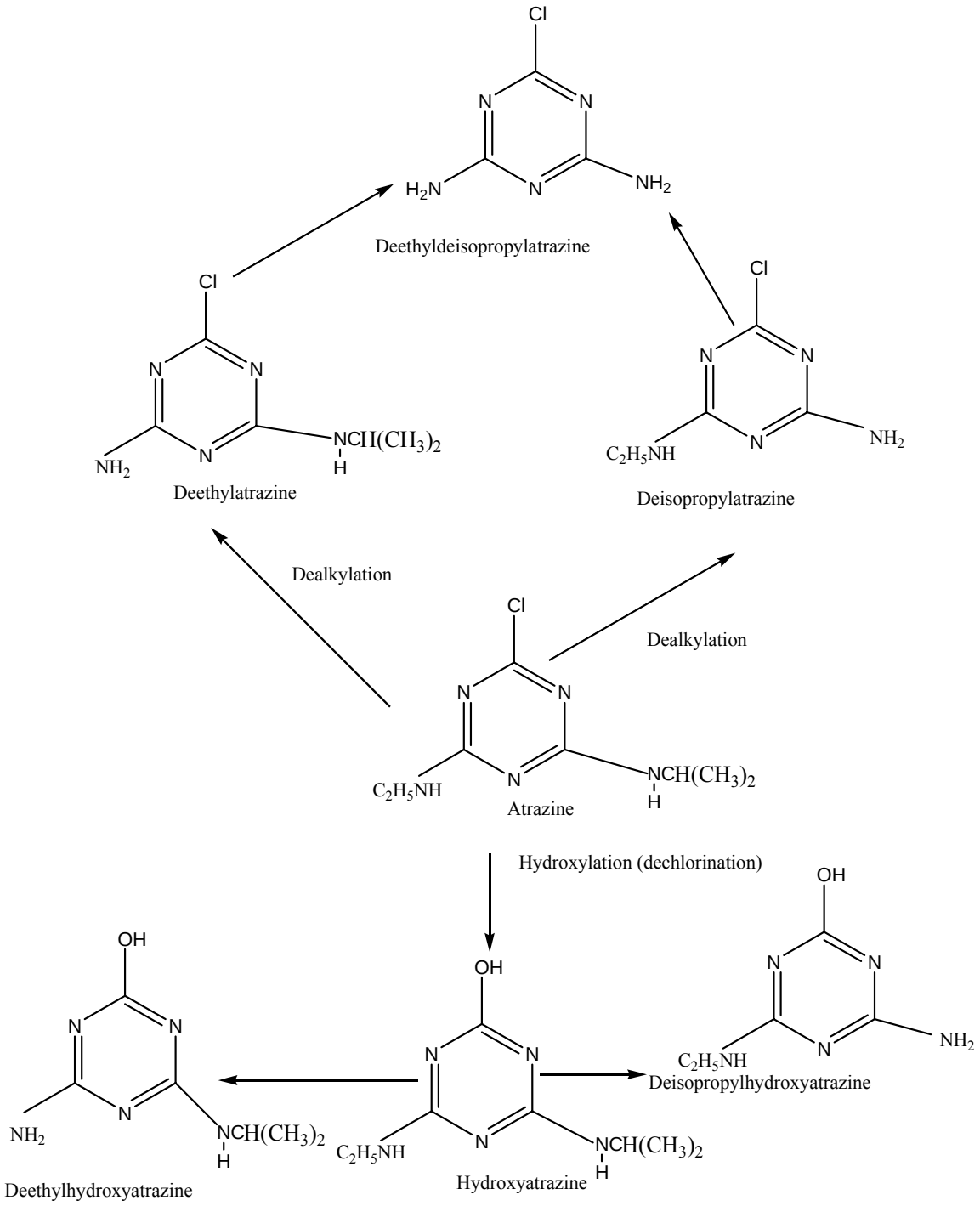


Fig. 4. Degradation Pathways of Atrazine [2, 36]

Sorption of herbicides on suspended particulates and bottom sediments has some effect on the fate of herbicides in water systems. When they are sorbed on sediments, their

persistence in water is increased. Sorption of herbicides on sediments in aquatic system can protect them from degradation in water [35]. The degradation mechanism for atrazine in salt marsh sediments is also considered to be insignificant, because salt marsh sediments have a pH range of between 7.2 and 8. 9 [36].

There is also a degradation reaction of herbicides occurring in plants. Two degradative reactions of triazine herbicides have been demonstrated to occur in the higher plants. One of these reactions involves hydroxylation of the ring in the number-2 position with dechlorination, demothoxylation and demethylthiolation. The other reaction is a dealkylation of the alkyl side chain in the 4 or 6 position. The rate of degradation of the triazine herbicides in plants varies greatly with different species. In tolerant plants, atrazine is readily metabolized to hydroxyatrazine while in susceptible or in sensitive plants, unaltered atrazine accumulates, leading to chlorosis and death. This is the basis for its selective use in several crops as well as the fact that a few weed control species are not controlled [2, 38].

1.4. Effect of Triazine Herbicides on the Environment

The required characteristics of recent pesticides include having a high effectiveness without causing environmental pollution or harmful effects, a high activity, low toxicity, high selectivity and being non-persistent [8]. But many of the pesticides that are used for crop protection and pest control are persistent and are causing environmental pollution. Thus the persistence of pesticide residues is often considered to be an acute environmental problem. Aquatic system is the one which is mostly affected by such chemicals. Therefore worldwide pesticide discharge into the aquatic environment is of an ongoing concern [19].

The risk of water contamination is greater for persistent pesticides when used in large quantities of location overlying shallow water tables [19]. Once these pesticides leave their point of use they change from being " crop-protection" and "pest control" chemicals to being environmental contamination that are suspected source of stress to aquatic plants and animals [4] . Aquatic organisms may die as a result of pesticide contamination, or

they may simply grow poorly, become more susceptible to disease, or become unsuitable for human consumption [37].

Recently there has been growing concern that certain environmental contaminants have a potential to disturb endocrine functions in exposed humans and wildlife. Disturbance by these endocrine disrupters may lead to impaired reproductive capacity and other toxicities related to sexual differentiation, growth and development [38]. Adverse effects induced by herbicide contamination were impacting a great variety of organisms and ecosystems, ranging from the primary producers to animals and humans [39], some of them are suspected to cause cancers, birth defects and interruption of hormone functions on human beings [6].

Herbicides are liable to affect non-target organisms, including micro-algae, leading to drastic ecological changes in the aquatic environment [40]. Since the herbicidal effect of triazine pesticides is based upon a hindrance of the photosystem II it may be hypothesized that in aquatic ecosystems, micro-algae are the first of the organisms to suffer from triazine contamination [18].

Some triazines exhibit carcinogenic properties and their use are controversial. Especially, simazine, atrazine and metribuzin are listed among 67 chemicals that are suspected to be endocrine disrupters by the Japan Environment Agency in 1998 [41]. Atrazine is also a common pollutant in surface and ground water and as soil contaminant [2]. The particular high mobility of atrazine in soil [23] and its potential contamination of surface and ground waters may represent a serious human health hazard because of the potential carcinogenic effects of s-triazines [2].

According to the European Union, threshold values for herbicides in water intended for human consumption are 0.1 μgL^{-1} for an individual herbicide and 0.5 μgL^{-1} for total herbicides [39]

After many years of use, atrazine residues may accumulate in subsoils and ground water and ultimately pose risks to humans consuming drinking water from contaminated ground water sources. When metabolite residues are combined with parent residues,

estimates of hazard levels to humans drinking contaminated water may be substantially higher. The biologically mediated degradation products, desethylatrazineand desisopropylatrazine, are known to have phytotoxicities equal to the parent compound [2, 36]

Atrazine has in general a negative impact on aquatic ecosystems and it has been reported to induce severe hormonal disturbances in amphibians [42] and in addition to its carcinogenic properties it has been recently reported to have long term reproductive and endocrine disrupting effects[7, 43,44].

Apart from causing contamination of soil, water and agricultural produce, there is also concern regarding the residual toxicity of herbicides to non-target crops. Studies on atrazine persistence and toxicity in Australian soils showed that sensitive crops such as sunflower may be damaged when grown in rotation after tolerant crops or if irrigated with contaminated water [45].

1.5. Effects of Contaminated Sediments in Water Ecosystem

1.5.1. Role of Sediments in Water Ecosystems

The particulate materials that lie below the water in ponds, lakes, spring, streams, rivers and other aquatic systems are called sediments. Sediments represent essential elements of aquatic ecosystems because they support both autotrophic and heterotrophic organisms [46].

Sediments support the production of aquatic organisms in several ways. For example hard bottom sediments which are characteristic of faster-flowing streams and are comprised largely of gravels, cobbles and boulders, provide stable substrates to which algae can attach and grow. Soft sediments which are common in ponds, lakes, and slower-flowing sections of rivers and stream, are comprised largely of sand, silt, and clay (i.e., fine sediment). Such sediments provide substrates in which aquatic macrophytes can root and grow. The nutrients that are present in such sediments can also nourish aquatic macrophytes. By providing habitats and nutrients for aquatic plants, sediments support

autotrophic production (i.e., the production of green plants) in aquatic systems. The role that sediments play in supporting primary productivity (both autotrophic and heterotrophic) is essential because green plants and bacteria represent the foundation of food webs upon which all other aquatic organisms depend (i.e., they are consumed by many other aquatic species) [46].

In addition to their role in supporting primary productivity, sediments also provide essential habitats for many sediment-dwelling invertebrates and benthic fish. Some of these invertebrate species live on the sediments (termed epibenthic species), while others live in the sediments (termed infaunal species). Both epibenthic and infaunal invertebrate species consume the plants, bacteria, and other organisms that are associated with the sediments. Invertebrates represent important elements of aquatic ecosystems because they are consumed by a wide range of wildlife species, including amphibians, reptiles, fish, birds, and mammals. For example, virtually all fish species consume aquatic invertebrates during all or a portion of their life cycle. In addition, many birds (e.g., dippers, sand pipers, and swallows) consume aquatic invertebrates. Similarly, aquatic invertebrates represent important food sources for both amphibians (e.g., frogs) and reptiles (e.g., turtles and snakes). Therefore, sediments are of critical importance to many wildlife species due to the role that they play in terms of the production of aquatic invertebrates [46].

Importantly, sediments can also provide habitats for many wildlife species during portions of their life cycle. For example, a variety of fish species utilize sediments for spawning and incubation of their eggs and larvae. In addition, young fish often find refuge from predators in sediments and/or in the aquatic vegetation that is supported by the sediments. Furthermore, many amphibian species hideaway into the sediments in the fall and remain there throughout the winter months, such that sediments provide important over wintering habitats [46].

In addition to providing habitats for many organisms, sediments serve as a time integrated sink of pesticide input from the catchments and therefore many toxic substances that are found at only trace levels in water can accumulate to elevated levels

in sediments. As such, sediments serve both as reservoirs and potential sources of chemicals of potential concerns to the water column [46].

Therefore, sediments play a variety of essential roles in terms of maintaining the structure (i.e., assemblage of organisms in the system) and function (i.e., the processes that occur in the system) of aquatic ecosystems.

1.5.2. Effects of Contaminated Sediments in Water Ecosystem.

Organic pesticides in a water system can be distributed into several compartments, depending on their water solubility. These compartments include water, aquatic organisms, suspended sediment and bottom sediment [35]. Pesticides, through the processes of erosion and streaming or drifting, can come in contact with surface water bodies and contaminate the sediments. The linkage of pesticides to sediment particles delays their migration and increases their persistence with potential risks to ecosystems. And this explains to a large degree why the toxicity of pesticides to wildlife is predominantly an aquatic problem in comparison with terrestrial ecosystems [5].

In recent years, concerns relative to the health and vitality of aquatic ecosystems have begun to reemerge. One of the principal reasons for this is that many toxic and bioaccumulative chemicals (such as metals, polycyclic aromatic hydrocarbons polychlorinated biphenyls, chlorophenols, and organochlorine pesticides, triazine herbicides, e.g. atrazine), which are found in only trace amounts in water, can accumulate to elevated levels in sediments [46].

Contaminated sediments have frequently been demonstrated to be toxic to sediment dwelling organisms and fish. As such, exposure to contaminated sediments can result in decreased survival, reduced growth or impaired reproduction in benthic invertebrates and fish. As a result of the effects of toxic and bioaccumulative substances, benthic organisms, fish, birds, and mammals can be adversely affected by contaminated sediments [46].

Contaminated sediments can also adversely affect human health and the human uses of aquatic ecosystems. Human health can be adversely affected due to direct exposure to contaminated sediments during wading or swimming in affected water bodies. Consumption of contaminated fish also poses a risk to human health. As such, contaminated sediments in freshwater ecosystems pose potential hazards to sediment-dwelling organisms (i.e. epibenthic and infaunal invertebrate species), aquatic-dependent wildlife species (i.e. fish, amphibians, reptiles, birds, and mammals), and human health [46] through food or water consumption [47].

Therefore, Pesticide residues in bottom sediments of streams draining agricultural lands can be a useful indictor of the entry of pesticides, from crop productions, into aquatic systems [47].

Investigation of the presence of pesticides in water bed sediments is important because bed sediments are the major sink for pesticides and persistent organic pollutants, in present and past uses, delivered to the aquatic system via various pathways [48, 49]. Analytical results from sediments have been used to evaluate pollutant sources historical trends and to predict water and thus exposure concentrations of biota in the aquatic environment [50]. Thus toxicological analyses of sediments can supply very important information about the state of health of water bodies. Therefore information on sediment quality conditions is of fundamental importance in the management of natural resources.

As a result of their potential toxicity to various aquatic organisms' separation, identification and determination of pesticide residues in sediment samples are necessary for the appropriate environmental remediation [51].

Because of their high risk of leaching into water systems and tendency to bind with sediments, the determination of triazine herbicide residues in different water-sediment samples is of special concern [52, 53]. This calls for the monitoring of sediment matrices for the levels of pesticides residues and their metabolites.

The analysis of semi-volatile and semi polar environment pollutants in sediments consists of several stages; i.e., isolation of the anlytes, extraction clean-up, and the final determination [54].

Many herbicides are semi-volatile, therefore they can be analysed by capillary gas-chromatography (cGC). Non-volatile herbicides can be derivatized with various derivatization agents before cGC analysis. As the volatility of herbicides may differ considerably, multiresidue analyses generally use thermal programming for the separation of the components of herbicide [55]

Thermolabile or non-volatile herbicides can be determined only by liquid chromatographic methods such as thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). Other analytical methods such as enzyme immunoassay and various electrophoretic techniques such as capillary electrophoresis (CE), micellar electrokinetic chromatography (MEKC) and isotachophoresis have also been used for the determination of herbicides residues [51, 53, 55, 56]. The methods actually used for the determination of trace amounts of triazines include gas chromatography and high performance liquid chromatography [51]

Environmental samples cannot be analyzed without some sample pre-treatment and preparation steps because they are too dilute and too complex. Consequently, analytical methodologies are needed which can detect pesticide residues and their transformation products (TPs) at ultra-trace levels in various types of complex environmental samples such as sediments. In the sample preparation process, solvent extraction has been traditionally used for most environmental samples. Other modern sample preparation techniques include: supercritical fluid extraction (SFE) [55], solid phase extraction (SPE) [57], solid phase microextraction (SPME) [58], microwave assisted solvent extraction (MASE) [59], accelerated solvent extraction (ASE) [60]

1.6. Pesticide Use in Ethiopia

About 80 % of the population of Ethiopia work within the agricultural sector and the whole country is dependent on the food production generated here. It has been recently

reported that a about 30-40% crop yields are lost to pests and diseases annually [61]. Agricultural intensification, including the increased use of pesticide in Ethiopia, is considered a viable option to overcome the problems of increasing population, shrinking farm sizes and food insecurity [61, 62].

Almost all pesticides that are used in Ethiopia are imported. Some come as donations from industrialized countries. Between the years 1983 and 1993 the import was approximately 3800 tonnes and the amount obtained on donation was about 203 tonnes annually. Of the imported pesticides 72% were insecticides, 25 % herbicides, 2.6 % fungicides and 1.3 % others [61].

Studies show that the state sector, with the former Minestry of state farms development, has been the major user of pesticides in Ethiopia [61]. Pesticide use in Ethiopia State farms is estimated at 7.76 kg/ha/yr, and less than 0.1 kg/ha/yr in smallholder farms [62].

There is also an expansion of flower industries in many parts of the country, mainly in Rift valley areas. Floriculture began in a limited way in 1984 has recently been expanded rapidly, occupying over 2000 ha [62]. The input of pesticides per hectare in floriculture is relatively high compared to cereals [63]. Most of pesticides used by the flower farms were not registered. The import of large quantities of pesticides without passing through the registration system of the Ministry of Agriculture and Rural Development presents a high risk for accumulation of future obsolete stockpiles. It also puts workers and nearby populations at risk of exposure to unregistered pesticides [62]. These farms are now very common in central rift valley areas and some other parts of the country. Because of a wide range of pesticides used by floriculture, it is becoming the main source of water pollution in central rift valley areas.

Other small–scale farms of cereals and grains that are owned by individual farmers are also very common in different parts of the country. These farms also use various types of pesticides to a certain extent. In addition, lack of awareness about possible hazards of these pesticides is rampant among the farmers. For example, Fig. 5 shows a farmer

preparing a pesticide for spray just adjacent to a river used for drinking purposes. This also enhances the extent of pollution of the water system.

Fig.5. A Farmer Preparing Pesticide not Far Away from Water Source which is Used by the Communities Living Around [62]

Herbicides are commonly used pesticide type to control various types of weeds in different types of agricultural crops. Of the herbicides, triazines are used in Ethiopia for the control of broadleaf weeds in wheat and barely, broadleaf and grass weeds in coffee, various weed species in sugar cane, complex weeds in maize and sorghum and annual weeds in maize, soybean and sugar cane [3]. During the usage of these pesticides, it is inevitable then that considerable amounts of applied pesticide can get ways to water systems by different routes.

On top of application of pesticides in agriculture and public health program, there are indications that stockpiles of obsolete pesticides left dumped in rotting bags and empty drums are contaminating surface and ground water supplies [64].

A recent study showed that the levels of triazine metabolites in Lake Awassa and its tributary river Tikur Wiha are well above the European Union residue limit in drinking water [65].

In spite of the widespread use of these pesticides, there is very little published information on the levels of these pesticides in different environmental compartments especially sediments. Particularly the pesticide residue status of sediments from the Rift Valley Lakes and their tributary rivers has not been determined in an organized manner. Therefore, the aim of this study is to undertake the preliminary processes for the determination of the residue levels of atrazine herbicide and its degradation products (Metabolites) in the sediment samples of Awassa and Ziway lakes.

2. EXPERIMENTAL

2.1. Chemicals and Reagents

Standards of atrazine and its metabolites (DEA, DIA and HA) used in this study were purchased from Dr Ehrenstorfer GmbH (Augsburg, Germany).

Stock standard solutions of 100 mg/L were prepared by weighing 0.5 mg of solid standards in weighing glass and dissolving in 5 mL volumetric flask in acetonitrile. Hydroxyl product of atrazine was first dissolved in small volume of 1.0 M HCl, to achieve complete solubility and diluted to the final volume with acetonitrile.

Working standard of the mixture of atriazine and its metabolites at lower concentration levels were prepared from the aliquots of stock standard solutions by appropriate dilution with distilled water. A series of standard mixtures with individual substance at the concentration levels of 1 mg /L, 0.5 mg /L, 0.25 mg /L , 0.125 mg /L, 0.0625 mg/L and 0.0313 mg /L were prepared for external calibration. All solutions were stored at 4 ^{o}C prior to analysis for future.

Analytical reagent Acetone; 99.5% pure, (Technopharmchem; India), cyclohexane for residue analysis and analytical reagent; 99.5 % pure (Technopharmchem; India) and doubly distilled ethyl acetate were used as extracting solvents. 99.5 % pure laboratory reagent NaCl; Labmerk chemicals (India) and 98 – 99.9 % pure anhydrous sodium sulphate, 37 % extra pure HCl (Riediel-deHail), 99.9% pure acetonitrile for HPLC ECHOMASOLV® far UV (Sigma Aldrich–GmbH), Filorisil; 60-100 mesh, n-Hexane; BDH (Poole, England) were also used.

2.2. Equipment

Magnetic stirrer with hot plate - model 04803-02 (USA), USA standard sieve ASTM E-11, A.S. S.H.O.M-92(1.7mm), Rotary evaporator; ROTAVAPOR -RE (BUCHI) Switzerland ROTAVAPOR-RE (Buchi Labortechnik, Flawil, Switzerland) and Analytical Balance; Mettler Toledo (Switzerland) , portable mV/pH meter; HANNA instrument(Portugal), DIHITHEAT oven; Abrera (Barcelona, Spain), Ultra Sonic heater (England) were used in the process of sample preparation.

2.3. Sediment Sampling

Sampling was carried out at two lakes in the Ethiopian Rift valley regions. These were Awassaa and Ziway lakes. There were a total of seven sampling sites, 4 sites at Awassa Lake and 3 sites at Ziway Lake. The locations of the sites are shown in Figs. 6 and 7.

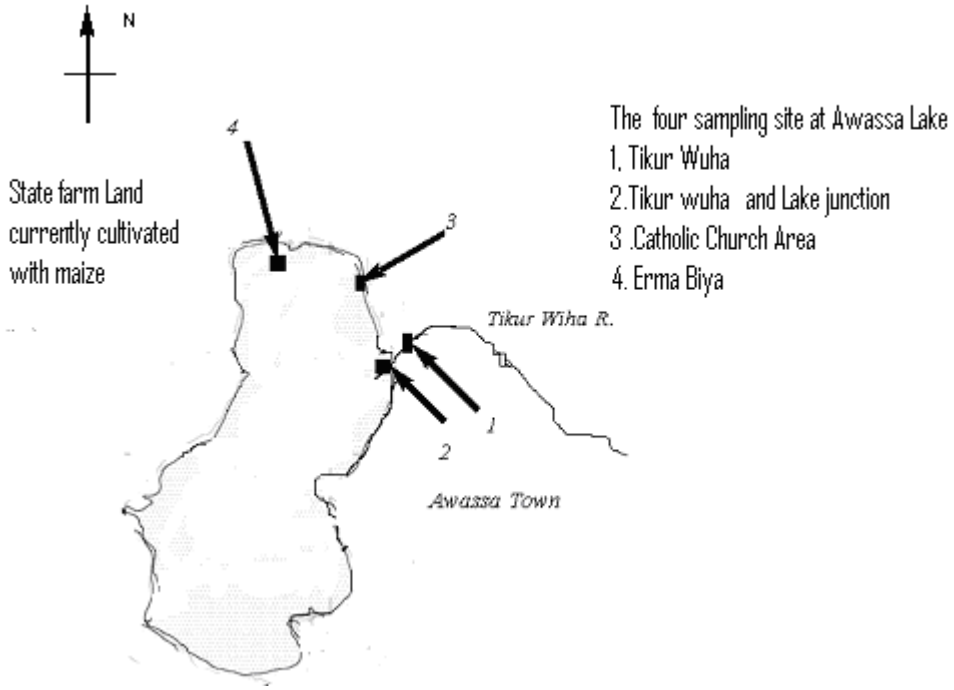


Fig. 6. Lake Awassa

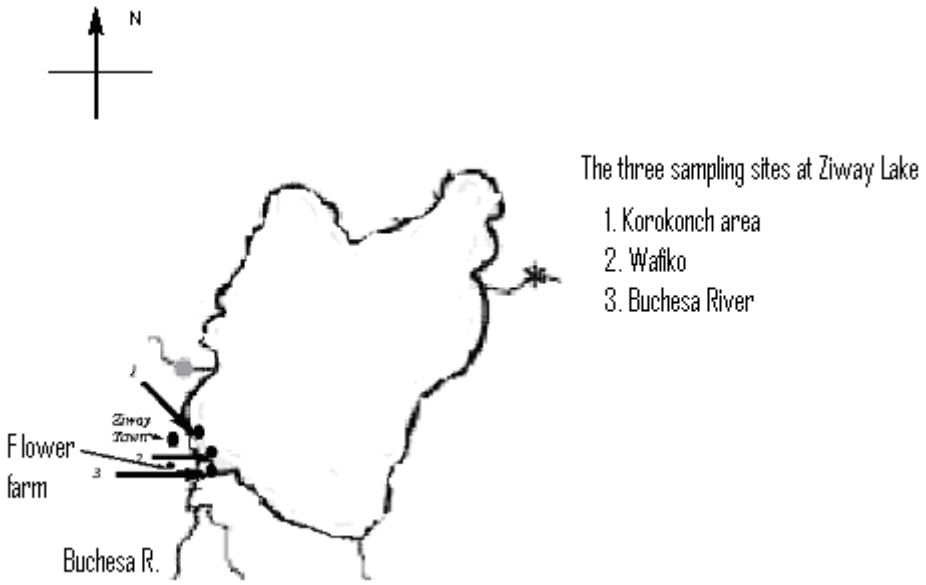


Fig .7. Lake Ziway

The eastern sides of the sampling sites at Aawassa Lake were Tikur Wuha River and the junction of the river and the lake. The third sampling site was at the northeast side of the lake. The fourth sampling site was at the north-west part of the lake, which is located at the state farm side, at which maize crop is currently cultivated.

From the sampling sites of Ziway Lake, one was the out flow of the lake i.e. Buchesa River, that is located at the southwestern side of the lake. The other two sites were located at the western part of the lake which is locally named as korokonch area and wafiko.

Table 2. Depth and distance from which the samples were collected from the lake water

A. Lake Awassa

Local name of Sampling site	Sample No.	App. Depth of water	Sampling point with in each site
Tikur Wuha	1	3 m	Lake body
	2	3 m	2 m away from the 1st
	3	30 cm	Shore side
Tikur Wuha and lake junction	1	1.40 m	Junction point
and lake j	2	1.40 m	3 m away from the jun. point.
	3	1.40 m	4 m away from the jun. point
Catholic church area	1	1. 10 m	Lake body
	2	10 cm	Lake shore
	3	1.10 m	3 m away from the lake shore
Erma Biya	1	80 cm	6 m away from the lake shore
	2	20cm	Lake shore
	3	1m	10 m away from the lake shore

B. Lake Ziway

Local name of Sampling site	Sample No.	App. Depth of water	Sampling point with in each site
Wafiko	1	1m	5 m away from the shore
	2	1.50 m	10 m from the shore
	3	1.50 m	4 m away from the 2nd
Buchisa R.	1	5 cm	River shore side
	2	2 m	Water body
	3	0.5 m	15 m from the 2nd
Korokonch area	1-3	1.80 m	10 m away from the lake shore to different directions

All samples were taken from 5 cm to 10 cm depth of sediment at the bottom of the water

Three samples were taken from each sampling sites of the two lakes. A total of twenty one sediment samples were collected manually on the mudflat of the two lakes; 12 samples from Lake Awassa and 9 samples from Lake Ziway. Depth of the lakes water and distance of the sampling points from point to another point are shown in tables 2A and 2B. After sampling from three positions at each site the sample was mixed thoroughly and three samples were taken from the mixed sediment samples.

As soon as the samples were taken from the lake water they were rolled with aluminum foil and put into polyethylene bags by labeling the required information on the bags. All samples were stored in ice cooled insulating box and transported to the laboratory.

Upon arrival at the laboratory, all samples that were taken from the same sampling sites were mixed and homogenized on aluminum foil and allowed to air dry at room temperature for 4-5 days. The sediment samples were stirred and mixed occasionally during drying period to ensure homogeneity. Then they were sieved through 1.7 mm (USA standard) mesh. This was followed by gentle grinding in mortar before transferring to plastic bags to be kept frozen (-18 ^{o}C) in a refrigerator until performing analysis.

Table 3. pH of the lake water during sampling, at each sampling site

Lake	**Site**	**pH**	**Site**	**pH**	**Site**	**pH**
Awassa	Tikur Wuha	6.35	catholic church area	8.38	Erma biya	8.40
Ziway	Korokonch	7.16	Wafiko	7.00	Buchesa R.	7.14

2.4. Extraction and Clean-up

The method of extraction was adapted from the guide line for SADC regions [66] with some modification. Sediment sample extraction was carried out by stirring, using magnetic stirrer (model 04803-02- USA). Triplicate subsamples of 10 g dry sediment

were taken from each sample container and mixed with 30 g of activated sodium sulphate powder in a mortar. The mixture was then ground until the sodium sulphate powder was flown freely. Other subsamples of 5 g sediment were also taken from each sample container and put in oven overnight for the determination of the moisture content of the samples. The percentage of water in the sample was determined first by weighing wet sediments, followed by drying at 105˚C, and reweighing to a constant dry weight. Dry weight determination of the sediment samples were carried out in triplicates at the time of sediment sample extraction.

A sub-sample mixed with anhydrous sodium sulphate was then extracted (stirred) successively four times with 50 ml, 20 ml, 20 ml, and 20 ml of acetone/cyclohexane (1:1 v/v) mixture for 15 min, 10 min, 7 min and 6 min, respectively, in 500 mL E-flask using magnetic stirrer. At the end of each extraction period the extract was separated from the sediment by decantation into E- flask and then filtered through filter paper into buchner flask using suction filtration.

After filtration, the filtrate was stirred with 200 mL of saturated sodium chloride solution, and allowed to stand for few minutes to salt out the water from the organic solvents, that is until the formation of phases, the aqueous phase and the organic phase. The two phases were separated by using 500 mL separatory funnel in 500ml E-flask. The aqueous phase was then extracted with 50 mL of ethyl acetate/cyclohexane (15:85 v/v) mixture. Again the aqueous phase and the organic phase were separated by the separatory funnel. The two organic phases were combined in an E-Flask containing 20 g of sodium sulphate. The sodium sulphate used was activated (heated) at 130 ^{o}C overnight in oven. The mixture was then shaked and left for about 15 min. The extract was then decanted through a plug of glass wool into an E- flask .The sodium sulphate residue remained in the flask was rinsed with 30 mL ethyl acetate/cyclohexane and decanted through the same glass wool plug. The final extracts were put in a refrigerator immediately after completion of the extraction.

The extraction procedure was followed by concentrating the extract to approximately 10 mL. This was carried out by evaporating organic solvents at 40-50^{o}C using rotary

evaporator. The concentrated extracts were transferred to clean vials using a pipette and stored in refrigerator until clean up.

Clean-up procedure

The florisil used for the clean-up was activated at 130^{o}C overnight prior to use. Glass column (I.D. 2.5 cm, length 32 cm) with Teflon stopcock was first plugged with glass wool. This was followed by packing with 4 g florisil, from the bottom, and 2 g sodium sulphate. The package was settled by tapping the column. The glass column was pre-conditioned by eluting 50 mL of n- hexane and then washed with small amount of acetone before loading the extract onto the column. When the solvent level was reached on top of sodium sulfate layer the stopcock was closed. This is to prevent further dripping. Then the yellowish colored extract was loaded drop by drop slowly onto the column by using pipette and allowed to flow by gravity through the florsil cartridge into100 mL of Erlenmeyer flask. The portion of extract retained by florisil was eluted with 50 mL of acetone/acetonitrile (1:1 v/v) mixture. The cleaned extract was put in the refrigerator at 4^{o}C until evaporating the solvent to concentrate and dry the extract.

3. DISCUSSION

3.1. General Observation

The samples collected from all sites of Awassa Lake were fine grain type sediment. The sediment samples from Tikur Wuha and the junction were brown in color and all the rest were gray type. Different sediment color is the reflection of its composition. Therefore, the brown color is may be due to the presence of primary oxidized iron. The gray color may also indicate the presence of sulfidized iron and pyrites**.**

Samples from Ziway Lake were of the same color as that of Awasa Lake, i.e. gray type. The sediment sample from the korokonch site was muddy type.

The final extracts obtained from all sediment samples were yellowish in color. This color was intensified after evaporating the solvent. Particulates were formed in some of the

extracts after few days' of storage in the refrigerator. As a result of the presence of this color, for the possible presence of macro-organic contaminants and other interferences a clean up step was included.

3.2. Moisture Content

It is usual to determine a moisture content of the each sample in order to express the result of analysis per dry mass of the sediment. Two portions of sediment samples were taken from each samples, one portion for extraction and the other portion for moisture determination. Percent moisture content was determined in all samples by drying an aliquot of each sample over night in drying oven set at 105^{o}C.

Percent moisture content, wet weight and dry weight of all samples were calculated by using the formula indicated below.

$$\text{Percent moisture content} = \frac{wetweight - dryweight}{wetweght} X100\%$$

Therefore, the percent moisture content of all samples was obtained as indicated in table

Table 4. Percent dry weight and percent moisture content of sediment samples

Study area	**Site**	**Av. Wet wt (g)**	**Av. Dry Wt (g)**	**Av. Moisture content(g)**	**Av. % moisture**	**% Av. dry wt.**
Awassa	Tikur Wuha R.	5.0232	4.9958	0.0486	0.9346	99.03
	Tikur W. and L. Jun.	4.9989	4.9606	0.0479	0.9554	99.08
	C. Church area	5.0345	4.9698	0.0647	1.2854	98.71
	Erma Biya	5.0452	5.0186	0.0337	0.6686	99.44
Ziway	Korokonch	5.0599	4.8576	0.2023	3.8096	96.00
	Wafiko	4.9927	4.9330	0.0597	1.1807	98.80
	Buchesa R.	5.0250	4.9709	0.0541	1.0499	98.92

As it can been seen from the table above percent moisture content of sediment sample from the krokonch area of Ziway Lake is the greatest of all; this is may be due to the mud content of the sample.

4. CONCLUSION

The experimental works carried out, in sampling and sample preparation, enables us to understand some chemical nature variations of the lake waters. For examples, Awassa Lake water is acidic when entering the lake and basic in the mass of the lake body. The acidity at Tikur Wuha junction may be attributed to some geological processes taking place upstream.

In addition, it was observed from our results of moisture content determination that the quantity of water adsorbed to the particles of sediments is negligibly low, about 1 %. Thus the possibility of further degradation of the target compounds after sampling and taking off of the bulk could be minimal. Furthermore, effectiveness of the clean-up technique in removing the possible particulate matters and other interfrents has been observed since the final extract appeared to lose much of its yellowish color.

However, it may not be possible to draw any final conclusion unless the analytical determination of the quantity of the residues is carried out. Our lab has recently received a new and modern HPLC system, which is to be installed soon. When the instrument is ready for use, completing the analysis of this preliminary project work may not require more than two weeks.

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