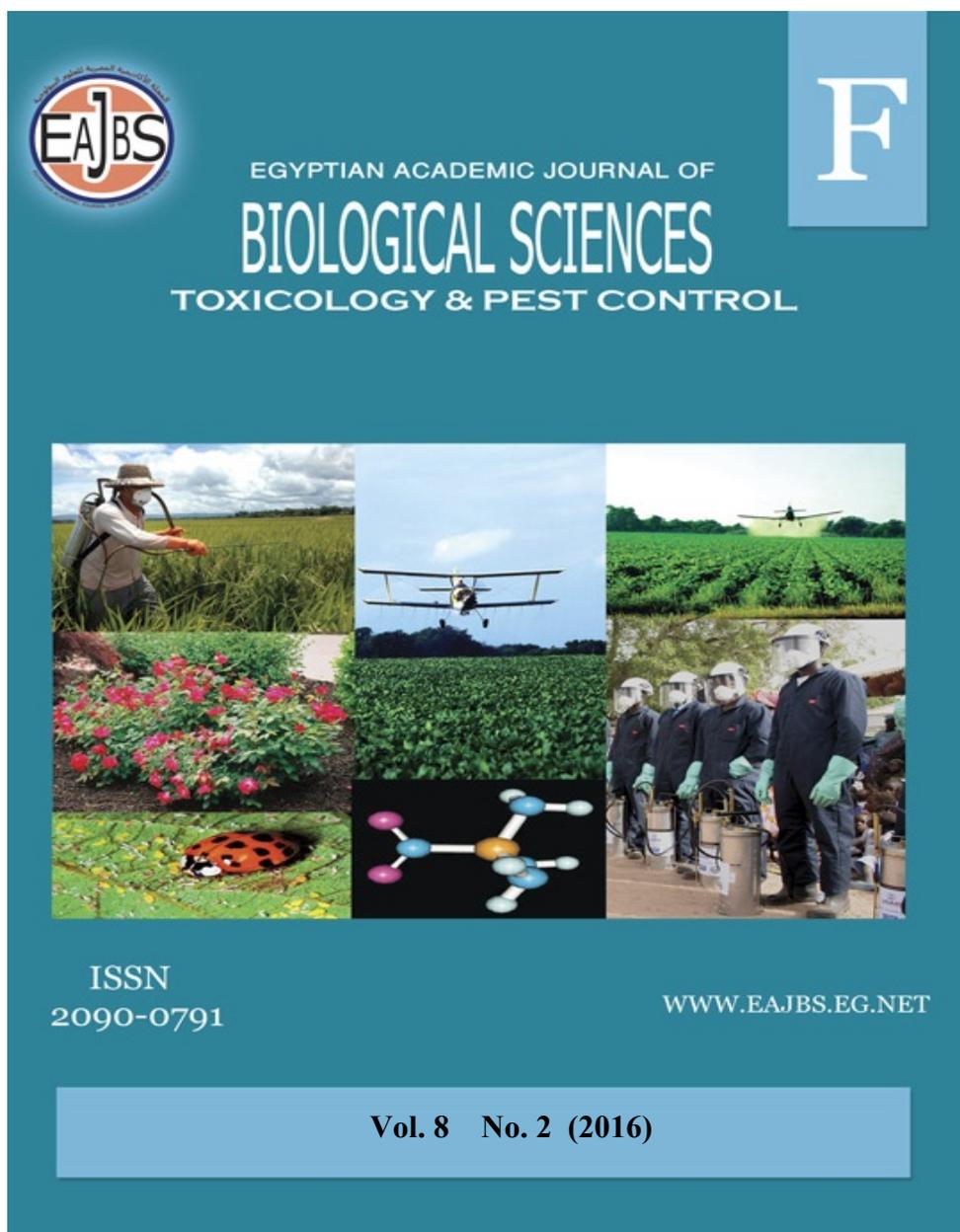


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Monitoring of Chlorpyrifos and Heavy Metals in Agricultural Area, Kerdasa Cairo

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ABSTRACT

Monitoring of organophosphorus and heavy metals was carried out in Abd El-Aal Canal, Kerdasa agricultural area, Cairo governorate to investigate the level of pollution with these contaminants in the aquatic ecosystems feeding farms. Samples were collected from Abd El-Aal Canal and analyzed by ICP-AES to detect cadmium, copper, iron, manganese, lead and zinc, and analyzed by GC to detect organophosphorus pesticides. Results revealed that the highest detected concentrations of manganese were (0.108 ± 0.008 mg/L) and lead (0.18 ± 0.002 mg/L) both were found exceeding the standard accepted limits. Only chlorpyrifos insecticide was detected in tested samples, and it was above the standard limits as well. As a result, the increased levels of chlorpyrifos, manganese and lead in Abd El-Aal Canal may cause stress to the aquatic ecosystem. The high level of detected contamination indicates potential danger on aquatic animals, farm animals, agricultural crops, and eventually human health. simple and economical methodology to remove pollutants from aquatic systems is recommended.

INTRODUCTION

The most vital natural resource that is needed by all life forms is water. The increase levels of pollutants in water may cause death of many life forms. For example, humans require water to replenish their internal system in order to survive. Also, humans cannot live for more than about a week without water (EPA 2013; Aulenbach, 1968). As a matter of fact the most abundant element in protoplasm (the fluid of the cell) is water. So, no organic matter would exist without water. Therefore, all life is dependent on clean, usable and accessible aquatic systems to survive (EPA 2013; Chaplin, 2001; Aulenbach, 1968).

The increase levels of pollutants in water may cause death or harm of many life forms-(Nourah *et al.*, 2015; Aulenbach, 1968). The increase of organic and inorganic pollutants in aquatic systems is expected from industrial and agricultural wastes (Ali *et al.*, 2013; Vasseur *et al.*, 2000). Also, the marine paints that protect boats from degradation discharge their effluents containing high amounts of heavy metals directly into the aquatic systems (Martin *et al.*, 2009).

The mean concentrations of phenol (0.04 ppm), lead (0.06 ppm), chromium (0.088 ppm) and Mercury (0.003 ppm) in Nile River at Rosetta Branch, Tahreer during 2009/2010 exceed the permissive limits; 0.02 ppm, 0.05 ppm, 0.05 ppm and 0.001 ppm for phenol, lead, chromium and mercury respectively (Osman and Kloas, 2010). Also, over the years, the Nile River is attaining many loads of industrial, agricultural and domestic wastes from several sources (Ezzat *et al.* 2012). There are many concerns as these pollutants will accumulate over the coming years. Also, aquatic environmental change is a sudden stress that may induce damage to molecular, cellular and organismal levels (Calabrese, 2006). Heat shock proteins (HSPs) also known as stress proteins are induced by stress (Soti and Csermely, 2007). This induction reflects severe alterations in the fish physiology and eternal molecular stability in response to the stress of the environment (Elnwishy and Sabri, 2009).

Aquatic reservoirs remain the ultimate sink of chemical pollutants emanating from anthropogenic activities such as agriculture, mining and industry. Freshwater biota undoubtedly is at risk from the adverse effects of these water pollutants and there is therefore, a need to monitor effects of these chemical pollutants in order to safeguard the health of aquatic biota. Aquatic reservoirs recently contain pollutants from anthropogenic activities such as agriculture, mining and industry. Aquatic organisms are at risk from adverse effects of these pollutants and there is therefore, a need to monitor these pollutants in order to remediate for safeguard and health of the aquatic environment (Nourah *et al.*, 2015).

The aquatic systems that are demands to living and industrial business are being contaminated each day by various anthropogenic activities such as

rapid growth of populations, urbanization and industrialization (Vasseur *et al.*, 2000). A major contamination found in waters due to industrial wastewater from mining, metal processing, tanneries, pharmaceuticals, pesticides, organic chemicals, rubber, plastics and wood products are heavy metal pollutants; Also, human activities such as burning of fossil fuel, mining and use of many chemical for crop growth contribute to the concentration of heavy metals in aquatic systems (Ali *et al.*, 2013). Then, these concentrations are spread by runoff water to other water sources resulting in pollution to be one of the major water pollutants to be concerned about (Srivastava and Majumde, 2008). These Pollutants can be toxic to animals and aquatic life at certain dosages (Agrawal *et al.*, 2010).

Organophosphorous is a group of about 250 chemical manufactured worldwide and about 140 of these products are pesticides; this group of pesticides is among the most toxic pesticides to vertebrate animals (Malhat and Nasr, 2013). Chlorpyrifos, also known as O, O-diethyl o-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate, is one of these chemicals which are highly toxic (WHO, 2009). Chlorpyrifos ($C_9H_{11}Cl_3NO_3PS$) is actually an insecticide that is a cholinesterase inhibitor with direct contact, stomach or respiratory action. It is applicable in agriculture, horticulture, viticulture, forestry and in a wide range of crops.

Therefore, efforts have been made by scientists to decrease or remove pollution in water resources (Akpoy and Muchie, 2010). Ex situ bioremediation can be applied to treat polluted water. The advantage of ex situ bioremediation is the certainty of the control treatment due to the uniformed in-vitro environment (Pavel and Gavrilescu, 2006). Organic waste can be very helpful for remediation of pollutants; chitosan

(organic waste) is non-toxic, biodegradable, biocompatible polysaccharide polymer that is very significant for heavy metal such as lead, copper, zinc, iron and chromium (Rathke and Hudson 1994), phenolic compound and pesticide removal (Rashed M., 2013). Pollution concentrations were reported to be significantly decreased by using the organic waste chitosan (Mohana-srinivasan *et al.*, 2014). Among recent approach is biological biosorbents such as fish scales to remove pollutants. The use of fish scale is attractive because they are inexpensive wastes found in markets daily (Prabu *et al.* 2012). Fish scales have mean metal concentrations in their body parts such as head, gills, intestine and the flesh (Jangu and Braich *et al.*, 2014).

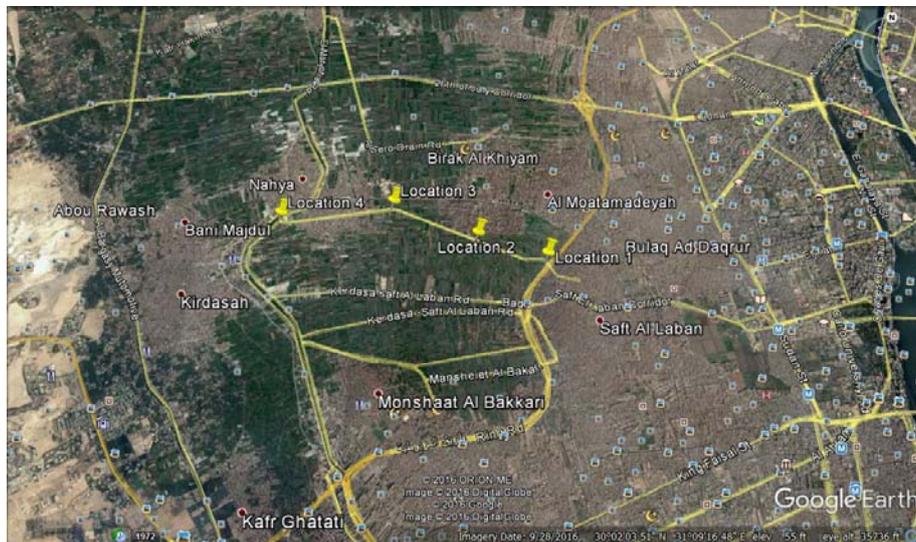
The present study aims to investigate concentrations of some organophosphorus compounds, and some heavy metals—at the canal of the studied

region as a case study to indicate the quantities of potential pollutants that might be supplied to the surrounding agricultural lands and farm animals since the canal is used for irrigation as well as the end point of drainage of the surrounding agriculture farms in the same time, and to indicate the level of pollution for the aquatic system of the canal.

MATERIALS AND METHODS

Sampling

Water samples were collected in glass bottles at 30 cm below surface. Samples were collected from Abd El-Aal Canal, Kerdasa agricultural area, Cairo during November, 2015 (Figure 1). A volume 300 ml of water samples were collected from each location. Sampling was made each 4 kilometer along 16 kilometers beside of the agriculture arena and the tested canal.



The samples were measured by ICP-AES (Inductively coupled plasma atomic emission spectroscopy (Model Ultima 2-Jobin Yvon, Horiba, France) to obtain concentrations of cadmium, copper, iron, manganese, lead and zinc. Gas chromatography (Model 6890, Agilent co., USA) was used to detect organophosphorus pesticides; ethop-

rophos, phorate, diazinon, dimethioate, pirimiphos methyl, chlorpyrifos, malathion, quinelphos, prothiophos, fenamiphos, ethion and trizophos, chlorpyrifos.

For analysis of heavy metal, the ICP-AES was adjusted at a wavelength (214.438 nm, 220.353 nm, 324.754 nm, 238.204 nm, 257.610 nm and 213.856

nm), cadmium, lead, copper, iron, manganese and zinc respectively. The flow was maintained at 0.48 liter per minute, pressure 2.5 bar and plasma temperature was maintained at 10,000 °C. A calibration curve was created by using blank (0.1ppm) and standard (1ppm and 5ppm). 5 ml of water sample was filtered to remove impurities at room temperature then placed in a sample tube to be aerosolized to the plasma in ICP-AES.

For organophosphorus analysis, Agilent SPE cartridges (ENVI-18, Agilent co., USA) were conditioned with 5 mL of Methanol (Sigma Aldrich, Germany), 5mL of acetone (Sigma Aldrich, Germany) and 5 mL of deionized water (Milli Q). Water samples (1L) were added to the cartridges to pass through at a flow rate of 10 mL/min. Cartridges were eluted with 5 mL of acetone and 5 mL of Deionized water. Then, samples were centrifuged at 1000-1500 rpm (112 RCF) for 5 min. The extract was collected by elution of cartridges with 3 mL of acetone.

Extracts were collected and evaporated to 2 mL using gentle blow of nitrogen (Imo, *et al.* 2007). 1 µl of prepared sample was injected in Agilent GC Model 6890, equipped with a flame photometric detector (FPD) - phosphorus filter. A fused silica capillary (PAS-1701, Agilent co., USA), column containing 14% cyanopropylsiloxane as stationary phase (30m length x 0.32 mm internal diameter (i.d.) x 0.25µm film thickness), was used for separation. GC injector and detector temperatures were conditioned at 240°C and 250°C; initial oven temperature, 160 °C for 2 min, raised at 5°C/min, Then, held at 230 °C for 15 min. The carrier gas was nitrogen at

3ml/min. The hydrogen and air were used for the combustion at 75 and 100 ml/min, respectively. The detection limit was 0.005 microgram/gram.

In vitro Experimental design:

Animal: Male Nile Tilapia (*Oreochromis niloticus*) fishes weighing 60 grams (2 months old) were obtained from Elkanater Fish Research Center, Cairo, Egypt during September, 2016. Fish were maintained following an approved protocol by the Animal Research Ethical Committee of Zewail City. Fish were stocked in equal numbers per each aquarium with 1 kg fish / 4 liters density. Aquaria were filled with dechlorinated water. Fish were conditioned for acclimatization for 5 days before conducting the experiment; water was adjusted at $27 \pm 1^\circ\text{C}$ and $\text{pH } 7.7 \pm 0.5$, and 150 ppm dissolved oxygen. Fish were fed on fish food pellets (40% protein) twice/day with 24 grams/day and sustained in natural photoperiod conditions.

Aquaria were distributed to 3 groups of 10 Nile Tilapia (*Oreochromis niloticus*) in each (Table 1). Each group was subjected to a different treatment of concentrations of the studied contaminants for 24 hours. Group 1 was treated with 2 ppm chlorpyrifos (Dow AgroSciences, USA), 0.005 ppm lead (Chem-lab, Belgium) and 0.006 ppm manganese (Chem-lab, Belgium). Group 2 was treated with 0.2 ppm chlorpyrifos (Dow AgroSciences, USA), 0.0005 ppm lead (Chem-lab, Belgium) and 0.0006 ppm manganese (Chem-lab, Belgium). Group 3 was used as control. Three fish samples were collected from each aquarium at the end of experiment interval. Results were then tested by SPSS for statistical analysis.

Table 1: Experimental design of *in vitro* experiment

Group	Treatment
G1	2 ppm chlorpyrifos 0.005 ppm lead 0.006 ppm manganese
G2	0.2 ppm chlorpyrifos 0.0005 ppm lead 0.0006 ppm manganese
G3	Control

RESULTS

Detection of organ phosphorus compounds and heavy metals Detected concentrations varied in correspondence with location (Table 2). Chlorpyrifos was the only detected organophosphorus compound in the water samples. The highest detected concentration was 8.3 ± 0.53 mg/L (Fig. 2) which exceeds the

standard limit of WHO (Table 2). Heavy metals were detected in concentrations of 0.11 ± 0.008 , 0.017 ± 0.001 , 0.113 ± 0.003 , 0.018 ± 0.002 and 0.031 ± 0.002 mg/L of manganese, copper, iron, lead and zinc respectively (Fig. 3).

Manganese was detected in high levels in location 1, 2, 3 and 4 compared to the standard limit (EPA, 2012).

Table 2: Detection of pollutants in the studied region

Pollutants	EPA Standard limit (mg/L)	Location 1 (mg/L)	Location 2 (mg/L)	Location 3 (mg/L)	Location 4 (mg/L)
Lead	0.015	ND	ND	0.018 ± 0.002	0.002 ± 0.001
Manganese	0.05	0.11 ± 0.008	0.06 ± 0.001	0.062 ± 0.002	0.06 ± 0.002
Copper	1.3	0.017 ± 0.001	0.013 ± 0.002	0.014 ± 0.003	0.013 ± 0.001
Iron	0.3	0.089 ± 0.001	0.089 ± 0.005	0.113 ± 0.003	0.102 ± 0.002
Cadmium	0.005	ND	ND	ND	ND
Zinc	5	0.031 ± 0.002	0.017 ± 0.003	0.021 ± 0.001	0.026 ± 0.004
Chlorpyrifos	0.001	8.53 ± 0.53	1.17 ± 0.07	1.14 ± 0.11	ND

ND: not detected

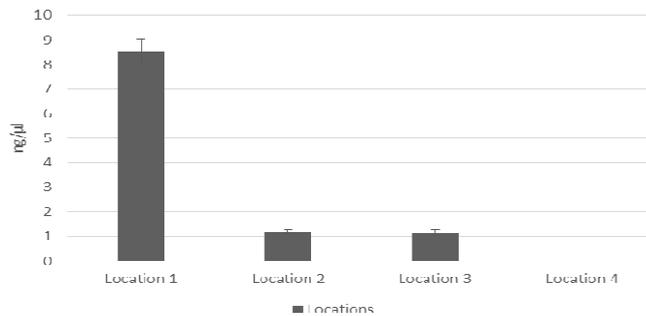


Fig. 2: Detected concentrations of Chlorpyrifos in the studied region

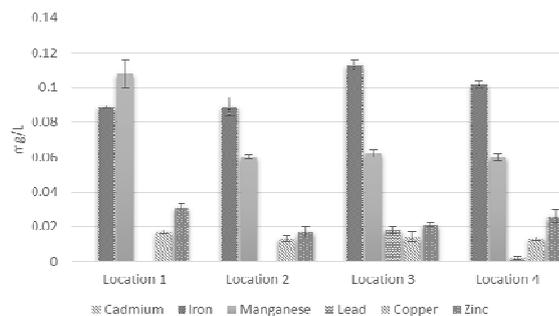


Fig. 3: Detected concentrations of Heavy Metals in the studied region

In-vitro Experimental design:

Each treated group resulted in 100% mortality of Nile Tilapia

(*Oreochromis niloticus*) as shown in Table 3.

Table 3: Effects of different concentrations of pollutants on mortality of Nile Tilapia (*Oreochromis niloticus*).

Group	Observation in 24 hours
G1	100% of mortality
G2	100% of mortality
G3	No mortality

DISCUSSION

Generally, the monitoring of Abd El-Aal Canal, Kerdasa indicated that concentrations of lead, Manganese and chlorpyrifos were detected in levels exceeding the acceptable standards limits. Unlike, copper, Cadmium, iron, and Zinc were detected in acceptable levels. However, all pollutants were decreasing gradually starting from location 1 to location 4. In fact, this maybe because location 1 was near the agriculture farms unlike location 4 which is 16 kilometers far from location 1. Also, the agricultural farms were gradually decreasing along the canal from location 1 to location 4 due to the encroachment of buildings. Accordingly, the studied pollutants were gradually diluted through water flow as the distance increases from the pointed sources of pollution (Afifi, R. *et al.*, 2016).

Concentration of Lead was only detected in location 3 and 4; 0.018 ± 0.002 , and 0.002 ± 0.001 mg/L respectively. This is mostly due to the effluent from the corroded and rusted service pipes plumbing the wastewater from the nearby building into the canal at locations 3 and 4 (EPA, 2016). In fact, human daily and anthropogenic activity may cause increase in naturally existing pollutants in the environment (Nourah *et al.*, 2015).

Also, manganese concentrations were detected in all locations above the standard limits; 0.108 ± 0.008 , 0.06 ± 0.001 , 0.062 ± 0.002 , and 0.06 ± 0.002

mg/L in locations 1, 2, 3 and 4 respectively. This may be due to the fact that the canal sides were surrounded by human activities near the agricultural land beside the canal. As a matter of fact, human activities such as metal smelting and organic wastes have been reported to increase manganese in soil of agricultural lands (Li P. *et al.*, 2014). In addition, manganese is present in soil and rock which can dissolve in ground or surface waters nearby (Nourah *et al.*, 2015). This indicates that manganese was present throughout the canal due to the soil (Howe *et al.*, 2004).

Chlorpyrifos was detected in high concentration (8.53 ± 0.53 mg/L) which is above the standard limits. The detected concentrations indicate that chlorpyrifos is mostly applied for treatment of crops in the agriculture activities near the canal. However, the application of pesticides treatment by aerial spraying which spreads the residues of the pesticides via air, leaching and runoffs after heavy rains was reported to result in supporting and increasing the presence of pesticides residues in the aquatic environment (Leistra *et al.*, 2010; Pimentel *et al.*, 2012). The effect of these residues in the environment can be even higher due to the presence of the contaminants caused by other resources which all have been detected in Egypt in several aquatic ecosystems; like pharmaceuticals and animal waste (Afifi, R. *et al.*, 2016; Elnwshy and Sedky, 2016; Elnwshy *et al.*, 2012), and organochlorine and organophosphorus

compounds (El-Sherif *et al*, 2009 a; El-Sherif *et al*, 2009 b; Elnwishy *et al*. 2007).

Since the concentrations of lead, manganese and chlorpyrifos were found to be toxic and above acceptable limits. They were tested in vitro to indicate the effect of their mixture on Nile Tilapia (*Oreochromis niloticus*). The results indicated of in vitro study on fish revealed full mortality of the tested fish population. This is mostly because of the effect of the mixture of pollutants. In fact, pollutants can cause harmful effect on aquatic organisms as well as other animals (Nourah *et al*, 2015) because pesticides are not species specific (WHO, 2009). Once the pesticide comes in contact with the target organism, it causes its harmful effect either immediately or on a long term exposure effect. The insects –which are the main target of pesticides-, are classified as simple species of animals, and since most animals have common characteristics, these similar characteristics enable non-targeted species to also be susceptible to the effect of pesticide (Agrawal *et al.*, 2010; Aktar *et all.*, 2009; Hurley *et al.*, 1998).

Additionally, the sublethal toxicity of lead is known to cause hematological and neurological effects such as impaired learning behavior (Hodson *et al.*, 1984; Adeyemo *et al*. 2010). Also, manganese causes hematological effects to fish (Sharma and Langer, 2014; Al-Akel *et al.*, 1998).

CONCLUSION

The high level of contamination in the canal with manganese, lead and chlorpyrifos suggests potential danger on aquatic animals, farm animals and agricultural crops which eventually becomes a source of human food. Less usage of pesticides and industrial human activities are recommended and further studies are required to investigate the presence of these pollutants in the river

Nile. Developing a simple and economical methodology to remove pollutants might be essential to recover the stress in aquatic ecosystems.

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ARABIC SUMMERY

رصد المواد كلوروبيريغوس والمعادن الثقيلة بمنطقة قناة عبد العال الزراعية بدراسة بالقاهرة

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١- الكيمياء كلية العلوم جامعة القاهرة

٢- مدينة زويل للعلوم والتكنولوجيا

٣- مدينة زويل للعلوم والتكنولوجيا

لقد تم رصد المواد الفسفورية الحيوية والمعادن الثقيلة بمنطقة قناة عبد العال الزراعية بدراسة بالقاهرة لاستكشاف درجة التلوث بهذه المركبات وتأثيرها على النظم الإيكولوجية المائية المستخدمة في امداد الاراضى الزراعية وتغذية حيوانات المزرعة بالمنطقة . وتم تجميع العديد من عينات المياه من المنطفة وتم تحليلها بواسطة جهاز (ICP-AES) للكشف عن نسبة الكاديوم و النحاس والحديد والمانجنيز والرصاص والزنك. كما تم تحليل المواد الفسفورية الحيوية بواسطة الكروماتوجراف الغازى (GC).

اثبتت النتائج ارتفاع معنوي بتركيزات المنجنيز (0.008 ± 0.010) والرصاص (0.002 ± 0.018) عن المعيار المسموح به دولياً. ووجد تركيزات عالية من مبيد كلوروبيريغوس المستخدم فى مقاومة حشرات المزارع فى العينات المحلله و كانت اعلى من المعيار المسموح به دولياً ايضاً.

تفيد هذه النتائج بهذه التركيزات المرتفعة التى تم الكشف عنه لكل من المانجنيز والرصاص مبيد كلوروبيريغوس قد تودى الى توتر هائل للنظام البيئي المائي بما فيها من كائنات مائية. وتفيد بزيادة معدلات استخدام هذه المواد فى المنطقة الزراعية مما قد يؤدى الى خطر كبير على الكائنات المائية، حيوانات المزرعة، المحاصيل الزراعية، وصحة الانسان فى نهاية الامر بالاستهلاك الأدمى. وكل ما سبق يشير الى ضرورة التوصل لطريقة بسيطة واقتصادية للتخلص من هذه الملوثات من البيئة المائية والحد من استخدام هذه المواد الخطرة .