Culiseta subochrea as a Bioindicator of Metal Contamination in Shadegan International Wetland, Iran (Diptera: Culicidae)

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Subject Editor: Carl Lowenberger

J. Insect Sci. 14(258): 2014; DOI: 10.1093/jisesa/ieu120

ABSTRACT. The quantity of some trace metals of mosquito larvae in Shadegan International Wetland from Iran was evaluated. Water, waterbed sediment, and mosquito larvae samplings were carried out from an urban site in the east of the wetland, using standard methods in December 2011. The identified Culiseta subochrea (Edwards) and Aedes caspius s.l. (Pallas) larvae, water, and waterbed sediment samples were analyzed for As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Pb, and Zn trace metals using standard preparation and isolation procedure. Result showed that the waterbed sediment and Cu. subochrea larvae are polluted with all trace metals investigated except As and Hg. The trace metals bioaccumulated in the Cu. subochrea larvae range from 31.78 at the lowest level for Cr to 3822.7 at the highest level for Cd. In a conclusion, this is the first report confirmed that Cu. subochrea likely used as a bioindicator to trace metal pollution in marine ecosystems in the world, especially wetlands.

Key Words: Culiseta subochrea, bioindicator, metal, Shadegan International Wetland

In recent years, the environment has been put to serious threat due to the discharge of harmful and toxic chemicals of various types (Sharma and Sharma 2010). Chemical analysis of the environment matrix such as water or sediment is the most direct approach to reveal the trace metal pollution status in the environment, while it cannot afford the powerful evidence on the integrated influence and possible toxicity of such pollution on the organisms and ecosystem. Biological monitoring or bio-monitoring is the systematic use of living organisms or their responses to determine the quality of the aquatic environment (Rosenberg 1998, Barbour and Paul 2010). Now it is recognized as one of the most valuable tools available in the arsenal of environmentalists (Mandaville 2002), which have advantages over physicochemical monitoring systems. It gives an indication of past and current conditions that readily reflects the abiotic or biotic state of an environment (McGeoch 1998, Hodkinson and Jackson 2005), whereas chemical and physical measurements provide data that primarily reflect conditions that exist when the sample is taken (Muralidharan et al. 2010). Chemical measurements are like taking snapshots of the ecosystem, whereas biological measurements are like making a videotape (Rosenberg 1998).

The utilization of invertebrates for assessing environmental conditions in aquatic ecosystems has thus long been recognized (Cairns and Pratt 1993). Invertebrates, in general, have growth rates and population turnover times lying midway between those of microorganisms and higher plants and animals. They have effective active and passive dispersal mechanisms that often allow wide dissemination and rapid recolonization of disturbed habitats. Finally, there is evidence that their responses are also indicative of changes in ecosystem function (Wallace et al. 1996, Hodkinson et al. 2002, Hodkinson and Jackson 2005). These their characteristics demonstrated that they can be suitable responsive indicators to changing environmental conditions of some pollution.

For aquatic metal pollution, the commonly used bioindicators mainly contained organisms including plankton, insect, mollusks, fish, plant, etc. (Zhou et al. 2008). In this regard, insects can be effective indicators because they have a short generation time (Anonymous 2009). Various insects living in or around the aquatic system can be used and monitored over time to assess the cumulative effects of environmental stressors (Zhou et al. 2008, Lokeshwari and Shantibala 2010) such as metal pollutants (Hare 1992). For example, Lithocerus niloticum (Hemiptera: Belostomatidae) was reported to be an efficient bioindicator to trace metal pollution in lakes (Sorour 2001). Mosquitoes may be the best known insects groups to man, because of their importance as larvae could be utilized in trace metal pollution studies. Although mosquitoes transmit diseases (Naficy and Saidi 1970, Saidi et al. 1976, Siavashi and Massould 1995, Maraghi et al. 2006, Azari-Hamidian 2007), their larvae such as Culiseta subochrea and Aedes caspius can be used as a valuable tool for aquatic environmental monitoring as bioindicators.

More and more attention has been drawn due to the wide occurrence of metal pollution in aquatic system (Duffus 2002), including Iran. Interestingly, small amounts of these elements are common in our environment and diet and are actually necessary for good health, but large amounts of any of them may cause acute or chronic toxicity (poisoning). Living organisms require varying amounts of trace metals but excessive levels can be damaging to the organism (Fargasova 1998, Annabi et al. 2009). Some trace metals may transform into the persistent metallic compounds with high toxicity, which can be bioaccumulated in the organisms and magnified in the food chains causing heavier exposure for some organisms than is present in the environment alone, thus threatening human health (Jin 1992). Various harmful effects including abnormal development of fetus, procreation failure, and immunodeficiency have been caused due to aquatic metal exposure (Chang et al. 2000). Although some metallic compounds can be strongly absorbed onto the suspended particles and sediments, they are released into the water under suitable conditions (Xu and Yang 1996). Finally, when introduced into the environmental water system, they may pose high toxicities on the aquatic organisms (Wu and Zhao 2006). Thus, monitoring and prevention of trace metal pollution is one of the hot topics in environmental researches (Zhou et al. 2008).
Wetlands take on characteristics that distinguish it as a distinct (Ramsar Convention Secretariat 2011) and the most productive among the world ecosystems (EPA 2010, Ornes et al. 2012). They naturally produce an array of vegetation and other ecological products that can be harvested for personal and commercial use (EPA 2012b). Wetlands also perform significant economic benefits that are extremely valuable to human society (Costanza et al. 1997). Therefore, protecting the wetlands in turn can protect our safety and welfare. A wetland system needs to be monitored over time in order to assess whether it is functioning at an ecologically sustainable level or whether it is becoming degraded (EPA 2012b).

Shadegan International Wetland in Khuzestan province, southwest of Iran, is considered to be one of the most wonderful natural attractions of the world because of its unique biodiversity and its linking to Jarahi river and Persian Gulf waters. It creates a suitable habitat for a large number of migratory birds that fly and arrive to this area every year (Hamidian and Harbach 2009). Among mosquito larvae identified, Cx. subochrea (Edwards) and Ae. caspius s.l. (Pallas) which had an enough numbers was used for trace metal isolation and analysis procedure.

Materials and Methods

Geographical Information. This study was conducted in Shadegan International Wetland area in Khuzestan province, southwest of Iran which is one of the 18 international wetlands registered on UNESCO’s Natural Heritage List. It is the largest wetland in Iran that covers an area of 537,700 ha, located 52 km far from Abadan and 40 km far from Ahvaz (the capital city of Khuzestan province) and surrounded from north to Shadegan city and Khor Daraq, from south to Bahmanshir river, from west to Darkhovien and Abadan road and from east to Khure-Musa and its surface is covered by great variety of vegetation. Its water supply is mainly through Karoun river. The area has a hot and humid climate and its coordinates are: 48° 17’– 48° 50’ E 30° 17’–30° 58’N.

Site Selection. Samples were collected from a site of the Shadegan International Wetland, which is located at the east of the wetland between Shadegan city and wetland where urban waste is released into the wetland.

Samplings. Because the precipitation and runoff waters have stopped by autumn and the rainfall of the next growing season has not started, the water that evaporates has reached its maximum in the end of summer. So it seems that trace metal pollutions are at their peak, during which the sampling was attempted.

Water and Waterbed Sediment Sampling. Sampling of water and waterbed sediment was carried out from the same selected sampling site in October and December 2011. Acid-washed watchglass were used for the grab collection and the samples were stored at 0°C in acid-washed (10% nitric acid) polypropylene tubes.

Mosquito Larvae Collection and Identification. Mosquito larvae collections were conducted from the same water and waterbed sediment sampling site in December 2011. Immature mosquito stages were collected in aquatic habitats using the standard larval dipping technique. The samples were collected with the minimum amount of water using sterilized polypropylene tubes. A sufficient amount of 96% ethanol was added and preserved at 0°C before taking to the laboratory. Specimens were washed with sufficient amount of 96% ethanol for removing any external contamination and identified using the morphology-based keys of Shahghudian (1960), Sirivanakarn (1976), Zaim and Cranston (1986), Harbach (1988), Reuben et al. (1994), and Azari-Hamidian and Harbach (2009). Among mosquito larvae identified, Cx. subochrea (Edwards) and Ae. caspius s.l. (Pallas) which had an enough numbers was used for trace metal isolation and analysis procedure.

Metal Isolation and Analysis Procedure

Mosquito Larvae Preparation Samples. A revised procedure by Lynch et al. (1988) was followed for mosquito larvae sample preparation and chemical analysis. The samples were oven dried for 24 h, then kept in desiccator for 24 h, the insect were acid digested with redistilled nitric acid and 30% hydrogen peroxide (H2O2). The dried samples were placed in 50 ml glass beakers with 10 ml of concentrated nitric acid (HNO3) for concentrations of 1.0 g. Each mixture was gently heated for 1 h, allowed to cool, then 5 ml of 30% H2O2 was added and heated gradually to boil (~10 min) and 5 ml of nitric acid was added. The solution was concentrated to 10 ml by heating. The cooled resulting solutions were passed through a 0.2-μm membrane filter into polyethylene bottles and diluted with double deionized water (DDI water) to various volumes within the linear range of the ICP-OES for analysis. The entire digestion process was done in a fume hood. Samples were stored at room temperature (about 25°C) until analysis. All glasswares and equipments were precleaned with 10% nitric acid and then rinsed with high-purity DDI water before and after each digestion process to avoid cross contamination of samples and biasing of the results.

Waterbed Sediment Preparation Samples. The waterbed sediment samples were oven dried at 60°C for 24 h in order to prevent the loss of possible volatile metallic compounds and to facilitate sample grinding and sieving. The samples were later homogenized by grinding in a mortar and pestle. The mortar, pestle, and sieve were cleaned before and after every sample with 10% HNO3 and rinsed with high-purity DDI water. Digestion and analytes extraction for ICP-OES analysis were performed using an acid mixture procedure (Creed et al. 1994). One gram of each sediment sample was precisely measured and transferred into a 50-ml glass beaker, then 4 ml of HNO3 (HNO3 1 part + DDI water 1 part) and 10 ml of HCl (HCl 1 part + DDI water 4 part) were added, and the solution was covered with a watch glass. The beaker was then placed on a hotplate for extraction of the analytes at an adjusted reflux temperature of 95°C. The sample was heated for 2 h while avoiding vigorous boiling of the solution (though very slight boiling could be tolerated) under a fume hood. The solution was then reduced to 10 ml by boiling, followed by cooling. The cooled solutions were passed through a 0.2-μm membrane filter into polyethylene bottles and diluted with DDI water to various volumes within the linear range of the inductively coupled plasma (ICP) for analysis. Samples were analyzed as soon as possible to minimize the effect of the various matrices on the stability of the diluted samples.

ICP-OES Analysis. The prepared laboratory samples for metals testing including As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Pb, and Zn were subjected to ICP-OES (Germany SPECTRO Company, Spectro atos Model) instrument to quantify the composition of the given samples. In this study, we used Trace CERT FLUKA analytical “Multielement standard solution 4 for ICP” (catalogue 51844, Lot & Pcode: BCBC8119 100976806) for all elements aimed in this study except Hg and a Certipur ICP standard of Hg [Order 1.70333.0100, Hg (NO3)2 in HNO3 10%] made serial dilutions and measured by the same ICP instrument (Sigma-Aldrich [Busch, Switzerland]). Then they compared with the water and the soil standards of the Environmental Protection Agency (EPA) and WHO (EPA 1993, EQS (MEGJ) 1994, EPA 2012a) using one-sample T test.
Results

Table 1 shows the instrumental detection limits, EPA water, and EPA or EQS soil standards, water, waterbed sediment, and mosquito larvae samples of all trace metals investigated by μg l⁻¹ and μg g⁻¹, and also mosquito larvae metal bioaccumulation index according to the water, waterbed sediment, and mosquito larvae sample preparation and analysis in the Materials and Methods. As shown in Table 1, the levels of all trace metals investigated in water samples were lower than the instrumental detection limits, except that the levels of Cr in both month samplings and Fe and Mn in October are above the EPA water standard by μg l⁻¹, which means that is the water that was polluted with Cr, Fe, and Mn trace metals investigated in this study. The levels of all trace metals investigated in waterbed sediment samples were above the EPA or EQS soil standards, except the levels of Cd and Hg that are lower than the instrumental detection limits, it means that the waterbed sediment was polluted with all trace metals investigated, except Cd and Hg. The levels of all trace metals investigated in the larvae of Cu. subochrea samples were above the EPA water and EPA or EQS soil standards by μg g⁻¹ except that the levels of Cr, Fe, and Mn trace metals investigated except As and Hg (Table 1).

Table 1 shows the instrumental detection limits, EPA water, and EPA or EQS soil standards, water, waterbed sediment, and mosquito larvae samples of all trace metals investigated by μg l⁻¹ and μg g⁻¹, and also mosquito larvae metal bioaccumulation index according to the water, waterbed sediment, and mosquito larvae sample preparation and analysis in the Materials and Methods. As shown in Table 1, the levels of all trace metals investigated in water samples were lower than the instrumental detection limits, except that the levels of Cr in both month samplings and Fe and Mn in October are above the EPA water standard by μg l⁻¹, which means that is the water that was polluted with Cr, Fe, and Mn trace metals investigated in this study. The levels of all trace metals investigated in waterbed sediment samples were above the EPA or EQS soil standards, except the levels of Cd and Hg that are lower than the instrumental detection limits, it means that the waterbed sediment was polluted with all trace metals investigated, except Cd and Hg. The levels of all trace metals investigated in the larvae of Cu. subochrea samples were above the EPA water and EPA or EQS soil standards by μg g⁻¹ except that the levels of Cr, Fe, and Mn trace metals investigated except As and Hg (Table 1).

Only the levels of Cr, Fe, and Zn trace metals investigated in larvae of Ae. caspius samples were above the EPA water and EPA or EQS soil standards by μg g⁻¹ but the levels of other trace metals including As, Cd, Cu, Co, Hg, Mn, and Pb were lower than the instrumental detection limits, which means that the larvae of Ae. caspius were polluted with Cr, Fe, and Zn trace metals investigated. Only the levels of As and Cd that were above the EPA water and EPA or EQS soil standards by μg g⁻¹ in waterbed sediment and larvae of Cu. subochrea, respectively, it means that waterbed sediment and larvae of Cu. subochrea were polluted with As and Cd, respectively. The level of Co was above the EPA water and EPA or EQS soil standards by μg g⁻¹ in waterbed sediment and larvae of Cu. subochrea, which means that waterbed sediment and larvae of Cu. subochrea were polluted with Co. The levels of all trace metals investigated in water, waterbed sediment, and mosquito larvae samples that were polluted are shown in bold (Table 1).

One-sample T test indicated that there was a significant difference between waterbed sediment and mosquito larvae samples of these polluted trace metals investigated (shown in bold in Table 1) and the EPA water and EPA or EQS soil standards (all P values < 0.05) (EPA 1993, EQS (MEGJ)1994, EPA 2012a).

In this study we defined an index named as bioaccumulation index, calculated by dividing Cu. subochrea and Ae. caspius larvae trace metals to waterbed sediment (Table 1). As shown in Table 1, the levels of all trace metals investigated except As and Hg (not shown) bioaccumulated in Cu. subochrea larvae ranges from 31.78 at the lowest level for Cr to 3822.7 at the highest level for Cd (Table 1), while at the first the Ae. caspius was not contaminated with the As, Cd, Co, Cu, Hg, Mn, and Pb, and at the second, the accumulation index of the contaminated Cr, Fe, and Zn trace metals is extremely low, ranging from 0.12 to 2.38 in comparison to Cu. subochrea, with its accumulation index being extremely high.

Discussion

Hodkinson and Jackson (2005) reported that insect has been used as bioindicators to indicate changing physical, chemical environment; the comparative quality or conservation value of habitat; and changes in the ecological status of the habitat with respect to time and place (Hodkinson and Jackson 2005). This article proved that Cu. subochrea is likely used as a bioindicator to trace metal pollution in marine ecosystems such as wetlands and confirmed that insects can be used to indicate changing chemical environment, particularly with respect to various forms of pollution (Hodkinson and Jackson 2005). However, it seems that Cu. subochrea can be used as a bioindicator to trace metal pollution in wetlands and any other aquatic ecosystems, as proved in this article which bioaccumulated in Cu. subochrea larvae ranging from 31.78 at the lowest level for Cr to 3822.7 at the highest level for Cd (Table 1).

Although, the levels of the Cr, Fe, and Mn trace metals investigated in the water are higher than the EPA water standard, it can be considered to be accumulated in the waterbed sediment and biomagnified there in the wildlife and animal tissues such as insect that lives in the wetland water, as shown in Table 1, and led to increased trace metal pollution because, finally they entered in the marine food chains, and biomagnified there after long periods.

As indicated in Table 1, it will be considered that the levels of trace metals shown in bold font was observed as above the EPA water and
EPA or EQS soil standards in the waterbed sediment and have been accumulated in the waterbed sediment and bioaccumulated in the Cu. subochrea tissues larvae as demonstrated in Table 1, which led to an increase in trace metal pollution, because they finally entered the marine food chains and biomagnified there after long periods.

As indicated in Table 1, only the larvae of Ae. caspius were polluted with Cr, Fe, and Zn trace metals investigated, whereas the waterbed sediment were polluted with all trace metals investigated except Cd and Hg. Therefore, larvae of the Ae. caspius are not recommended as a bioindicator to trace metal pollutions, because at the first it is not contaminated with As, Cd, Co, Cu, Hg, Mn, and Pb trace metals and at the second, the accumulation index of the Cr, Fe, and Zn is extremely low in comparison to Cu. subochrea, with its accumulation index being extremely high.

This study indicate that chemical analysis of the environment matrix such as water, sediment is the most direct approach to reveal the trace metal pollution status in the environment, while it cannot afford the powerful evidence on the integrated influence and possible toxicity of such pollution on the organisms and ecosystem (Muralidharan et al. 2010), but demonstrated that trace metals can be bioaccumulated in the organisms such as Cu. subochrea tissues larvae. In a study conducted by Mireji et al. (2008), the concentrations and distribution of cadmium, chromium, copper, iron, lead, manganese, and zinc in mosquito larval habitats in urban Kismanu and Malindi, Kenya and their effect on the presence of Anopheles gambiae, Ae. aegypti, Culex quinquefasciatus, and An. funestus larvae were investigated. Results revealed that copper was positively associated with the presence of Ae. aegypti, and lead was associated with the presence of An. gambiae and Ae. aegypti in urban Kismanu. Also Kitvatanachai et al. (2011) reported that Cx. quinquefasciatus larvae can bioaccumulate the metal and can potentially serve as a biomarker of lead contamination to complement conventional techniques. Consequently, these works confirm that some mosquito larvae can be used as a valuable tool for aquatic environmental monitoring as bioindicators.

In a conclusion, this article confirmed that Cu. subochrea is likely used as a bioindicator to trace metal pollution in marine ecosystems such as wetlands, as has been proved due to its widespread occurrence in different regions, easy collection, and identification. This is the first report to confirm that Cu. subochrea can be used as a bioindicator to trace metal pollution in marine ecosystems in the world, especially wetlands.

Acknowledgments

This study is a part of the H.N. PhD thesis in Medical Entomology and Vector Control from Department of Medical Entomology and Vector Control, School of Public Health, supported by Tehran University of Medical Sciences (6477), Tehran, Iran. We are extremely grateful to Prof. Alineza Medaghiyon and Prof. Simin Naseri for their support to carry out this research. We would also like to thank Prof. Yavar Rassi for his true support to carry out this research.

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