Egypt .J. Aquat. Biol. & Fish.,Vol.5,No. 2: 129 - 146 (2001) *ISSN 1110 – 6131*

ANISOPS SARDEUS SARDEUS HERRICH-SCHAEFFER (HETEROPTERA: NOTONECTIDAE) AS A BIOINDICATOR OF HEAVY METALS IN WASTEWATER TREATMENT PLANT AT ISMAILIYA, EGYPT

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Key words: *Anisops sardeus sardeus,* bioindicator, heavy metals, wastewater treatment, Ismailiya.

ABSTRACT

Heavy metal contents of *Anisops sardeus sardeus* Herrich-Schaeffer collected from polishing and facultative lagoons in Ismailiya Wastewater Treatment Plant, Egypt, were analyzed by AAS. This species is studied for the first time as a biomonitor of heavy metals in water and sediment. The average concentrations of heavy metals in insect tissues found in facultative lagoon vs. the polishing lagoon were: Zn 197, 147.4; Cd 2.7, 1.5; Cu 28.2, 13.7; Co 37.7, 34.4; Pb 59.7, 57.8; Ni 36.9, 25.1 µg/g dry weight respectively. With the exception of lead, the differences in concentrations of each metal were statistically significant. These concentrations of metals in the insect tissues corresponded to their concentrations in the two lagoons. The present study confirmed that *Anisops sardeus sardeus* from polishing lagoon is an excellent accumulator of Co and Pb as their concentrations reached 34.4 and 57.8 µg/g dry weight, respectively, than in water (9.1 and 41.0 mg/l) and sediment (29.0 and 42.0 µg/g) samples. The bioaccumulation factor was 12.3, 1.5, 1.3, 3.8, 1.4 and 0.3 for Zn, Cd, Cu, Co, Pb and Ni, respectively. It is, thus, concluded that *Anisops sardeus sardeus* could be a very suitable accumulative bioindicator for the monitoring of heavy metals in surface water of wastewater treatment plant.

INTRODUCTION

Heavy metals can be a serious water quality concern because of their toxicity, persistence and potency. Metals can accumulate in sediments and magnify in fish and shellfish tissues. Sources of heavy metals and toxic substances include storm water runoff, solid waste disposal areas, household and industrial uses, agriculture and wastewater discharges (ATSDR, 1992).

Metals from industrial discharges are deposited in different components of the aquatic ecosystem, such as water, suspended particulate matter, sediment and biota. In general, the greatest pools or sinks of chemicals in the aquatic environment occur in the sediment (Adams *et al.*, 1992). A great amount of money is spent in the determination of heavy metals in water and sediment by chemical analysis. However, this gives poor information about the toxicity and bioaccumulation of the metals in the tissues of the aquatic organisms (Giesy & Hoke, 1990).

Heavy metals, even at very low concentrations, can be strongly accumulated through the food-web in aquatic systems (Nummelin *et al.*, 1998). Many aquatic organisms spend the major portion of their life-cycle in water, living on or in sediments and may provide a pathway for chemicals to be accumulated and consumed by higher organisms, including humans (Bervoets *et al.*, 1994). As a consequence, analysis of the metal content of aquatic insects is necessary in the quality assessment of surface waters, providing information about the bioavailability of metals (Gower & Darlington, 1990).

However, insects are fairly little used as bioindicators of heavy metal pollution (Spehar *et al.*, 1978 & Luther, 1993). Individuals of many species are not easy to collect in quantities large enough or there is short seasonal supply. Often their origin is unknown due to their flying ability. In many cases also a taxonomic expert is needed to determine the species. Notonectidae overcome many of the above problems as they are predators (Truxal, 1979) and thus prone to bioaccumulation. They are often easy to collect in large quantities, species determination is fairly easy on species level (Schuh & Slater, 1995) and even adults do not disperse for long distance (Gittelman, 1974 & Fairbain, 1986).

The objective of the present study is to investigate if concentrations of heavy metals (Zn, Cd, Cu, Co, Pb, and Ni) in the aquatic insects could be related to metal levels in water and sediment. Therefore, the relationship was studied between the metal levels in water and sediments and the concentrations were determined in *Anisops sardeus sardeus* (Heteroptera: Notonectidae) collected from Ismailiya Wastewater Treatment Plant.

MATERIAL AND METHODS

Species studied:

Anisops sardeus sardeus is the most common species of a cosmopolitan family Notonectidae (Linnavuori, 1964); and is found all the year round in Egypt (Saleh Ahmed *et el.*, 1992). It is a backswimmer because it swims upside down, usually near the surface of water. It is a predaceous insect that can help in biological control measures (Tawfik *et al.*, 1986). It lives in lentic habitats or the quiet parts of the streams (Polhemus, 1995). Line drawings were prepared with the aid of a Camera Lucida fitted on a Wild microscope. Adults were collected from Ismailiya Wastewater Plant during August 2000.

Study area:

The area of study was wastewater treatment plant established in 1996 at Ismailiya by funding from U.S.A Aid and the Egyptian government; and was designed to serve the population of Ismailiya City.

The wastewater collection system serving the city of Ismailiya consists of gravity collector and interceptor sewers, which transport flow to four major pumping stations. The major pumping stations are designed to discnarge through force mains to a junction box and gravity interceptor, which flows to Abu Attwa pumping station. Wastewater is pumped from the Abu Attwa pumping station to the new wastewater treatment plant site at Serapuem through approximately 11 Km of new 1200-mm diameter force main. The new treatment plant site is located approximately 15 Km of the central urban area of Ismailiya.

Effluent from the plant is discharged to El-Mahsama drain through the plant out-fall line. The plant out-fall consists of a 1700-mm diameter pipeline through which wastewater flows by gravity to an outfall structure located east of the wastewater canal. The total length of the plant outfall is approximately 9.600 meters.

The preliminary treatment of wastewater at the headwork facilities of the station includes flow measurement, screening and grit removal. Effluent from the grit basins flows into the treatment lagoons (aerated lagoon, facultative lagoon and polishing lagoon). Aerated lagoon is responsible for complete mixing and increasing the oxygen supply of the wastewater inside the pond. The facultative lagoon helps the heavy solids to settle down on the bottom of the pond. The polishing lagoon allows additional solids to settle. Sludge is removed from the facultative and polishing lagoons on a regular basis by self-propelled dredges.

Sampling:

Insects were collected by sweeping the water with a net. Water samples were filtered through a 0.45 µm millipore membrane filter and acidified (nitric acid, pH 2.0). Sediment samples were sieved using a 500 µm-mesh sieve and stored in polyethylene bags. All samples (insects, water and sediment) were stored at 4 °C till the time of analysis.

Analytical procedures:

All collected insects were first killed by deep freezing and dried at 60 °C in plastic tubes in a drying chamber for 10 hours and then stored at room temperature. Before the chemical analysis, samples were dried overnight at 105 °C. The animal samples were weighed (0.25-0.5 g) in glass test tubes; 3.5 ml of concentrated HNO_3 and 10.5 ml of concentrated HCl were added, and left for 24 hours. The samples were then heated with more addition of concentrated HNO_3 till the entire brown fumes disappeared. The samples were filtered and diluted with distilled water to 25 ml (Nummelin *et al.*, 1998). The water samples were analyzed by mixing 25 ml of water with 1 ml of concentrated HNO_3. They were heated to concentrate the volume to 5 ml, then the samples were diluted with distilled water to 25 ml (APHA, 1989). The sediments were dried at 105 °C and digested in a mixture of HNO_3 and $HOCl_4$ (4:1) (Moore & Chapman, 1986).

The heavy metals concentrations (Zn, Cd, Cu, Co, Pb, Ni) in water, sediment and in extracts of insects were measured by flame atomic absorption spectrophotometer, AAS (Perkin-Elmer 2380).

Results are expressed in mg/l (ppm) for metal concentration in the water samples and in µg/g dry weight (ppm) for heavy metals and insect tissue. The distribution behavior of heavy metals between the water, sediments and biota can be expressed as bioaccumulation factor (BAF). This BAF is usually computed as: BAF= Cs/Cw (Verhaar, 1995), where: Cs is the concentration in biota and Cw denoting aqueous concentration.

Dissolved oxygen was measured with a polarographic oxygen electrode system (WTWOX191/EO90), BOD with YSI5905 BOD Probe - model 58, COD with COD reactor-HACH test kit 41100-O4. PH was measured with a glass electrode (ingold 104573002, WTW pH 90). TSS (total suspended solids) were measured by a portable meter WTW LF91.

Statistical analysis:

The statistical significance of the differences between means of facultative and polishing lagoon values for each metal were tested using one-way analysis of variance, ANOVA with Tukey's test (Bailey, 1981).

RESULTS

Diagnosis of the studied species

General body form (Fig. 1) long and slènder, dorsum strongly convex, venter concave. Claval commissure with a large sensory pit (Fig. 1). Labium with an apposing "prong" (Fig. 2). Male foretibia with a stridulatory comb located proximally on interior surface (Fig. 3). Metathorcic scent glands are completely absent. Male genitalia is characterised for each species (Fig. 4).

Insect tissues, water and sediment analysis:

The physico-chemical parameters of the study area were presented in Table 1. The temperature average was 30.6 °C and pH ranged from 7.6 to 8. The levels of six heavy metals (Zn, Cd, Cu, Pb, and Ni) in the insect tissues of *Anisops sardeus sardeus,* in water and in sediment were evaluated.

Insect samples taken from the facultative lagoon had significantly higher heavy metal concentrations than other samples collected from the polishing lagoon (Table 2). This was related to the concentration of heavy metals in water and sediment of both sites (Tables 3 and 4). Zinc was accumulated in the insect tissues to a very high concentration in the polishing and facultative lagoons (147.4 and 197.1 μg/g dry weight respectively) compared to 12.0 and 148.0 mg/l water of the two sites, respectively. In the polishing lagoon, cadmium was not detected in water samples (Table 3), but it was detected in the insect tissues (1.5 μg/g dry weight). However, it was detected in water samples of the facultative lagoon (3.8 mg/l) and in collected insects (2.7 μg/g dry weight). The highest concentration of copper was found in sediments of the polishing and facultative lagoons (22.4 and 62.6 μg/g) compared to water (10.3 and 27.5 mg/l) and insect tissues (13.7 and 28.2 μg/g dry weight), respectively. In the polishing lagoon, insect tissues contained higher concentrations of Co and Pb (34.4 and 57.8 μg /g dry weight) compared to water (9.1 and 41.0 mg/l) and sediment (29.0 and 42.0 μg/g) respectively. Nickel concentration in the insect tissues (Table 2) was much less than in water and in sediment of the polishing and facultative lagoons (Table 3 and 4). The concentrations of zinc and lead in the insect tissues were higher than the other heavy metals in both lagoons (Table 2).

The calculated bioaccumulation factor (Table 5) indicated that the accumulation factor of Zn in insect tissue from polishing lagoon was 12.3, while that of Co was 3.8. In other words, the value of bioaccumulation factor of Zn was approximately 4 fold higher than Co. The bioaccumulation factors of other metals were much lower. In general, the bioaccumulation factors of all heavy metals were higher in the polishing lagoon than in the facultative lagoon; as the concentration of heavy metals in water samples decreased in the polishing lagoon.

DISCUSSION

Monitoring of the quality status of natural waters has traditionally been carried out using physicochemical techniques, but there are attempts that have been made to include the biological responses of organisms in monitoring systems for detection of pollution in aquatic environment (Diamond *et al.,* 1988). Direct analysis of water is not an economical method for monitoring levels of heavy metal pollutants because they are usually present in water in very low and rapidly fluctuating concentrations; and therefore, sampling needs to be frequent (Herve *et al.,* 1988).

According to the results obtained in the present study, the heavy metals in *Anisops sardeus sardeus* tissue could provide a powerful monitoring tool. The present work confirmed that *Anisops sardeus sardeus* from polishing lagoon is an excellent accumulator of Zn, Co and Pb.

In the present study, the concentrations of heavy metals (Zn, Cu and Pb) in water samples of the polishing lagoon were 12, 10.3 and 41.0 ppm, respectively; whereas, the maximum concentration level of these metals is recommended by Federal Drinking Water Standard to be 10, 1.3 and 0.015 ppm, respectively. Also, it is recommended that if 1.3 ppm copper or 0.015 ppm lead is detected in tap water, the water works is required to modify its chemistry to make it less corrosive (Internet I). The maximum allowable concentration of nickel in fresh water is 10 µg/l (Internet II); while in the present study, nickel concentrations were 25.1, 39.0 and 86.2 ppm in insect tissue, water samples and sediment of the polishing lagoon respectively (Tables 2, 3 and 4). Effluent from the plant may be used for irrigation and therefore, should containe the allowable levels of heavy metals. Most of the heavy metals stick to the soil particles and move to underground water (ATSDR,1992).

Although Cd metal was not detected in the water samples of the polishing lagoon, *Anisops sardeus sardeus* is still able to accumulate 1.5 ppm of Cd in their tissue. Cadmium, however, was detected in higher concentration in the sediment than in the biota or water samples. The Cd concentrations observed in this study are not as high as observed in water striders in Hanko Penisula (6.5 µg/g) (Nummelin *et al.,* 1998), but much higher than in Trichoptera larvae in acidificated lakes in Finland (0.5-2 µg/g) (Verta *et al.,* 1990).

At some stations that are contaminated with heavy metals, water column bioassay did not reveal any toxicity, while sediment and especially in situe bioassay, reveals. Because more persistent chemicals tend to accumulate in sediments, concentration of heavy metals was determined. The highest concentrations of Zn, Cd and Cu were detected in the sediment compared to their concentration in water and insect tissue of the polishing lagoon (Tables 2, 3 and 4). This is because Zn, Cd, Cu and Pb have specific gravity greater than that of water so, the levels of pollutants in the sediment are higher than in water (ATSDR, 1992). Water chemistry of the system controls the rate of adsorption and desorbtion of metals to and from sediment. Metals may be adsorbed to sediment if the salinity of water decreased, redox potential increased or pH increased (Kwapuli ski *et al.*, 1991 & Ibrahim *et al.*, 2000).

The bioaccumulation factor (BAF) gives an idea of the accumulation capacity of each metal by *Anisops sardeus sardeus*. Bioaccumulation of trace organic contaminants depends principally on simple physicochemical processes, which are based on the partitioning of these contaminants between aqueous and non-aqueous phases. This relationship is evident in the correlation between the water soluble of an organic contaminant and its net uptake by aquatic organisms. This explains why the bioaccumulation factors of Zn, Co and Cd in insect tissue were higher than the other metals. High concentrations of these heavy metals in insect tissue may be partially explained by their higher content in samples or via both particles used as food and directly via water passing over the mouth parts.

As shown in the present results, heavy metals can not be monitored through water samples. Monitoring programs based on water sampling have, therefore, to be replaced by biomonitoring programs. According to the present results, it seems that *Anisops sardeus sardeus* could be a suitable bioindicator for heavy metal studies.

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Table 1. Selected water quality variables of the study area (August, 2000)

Parameter (mg/l)	**Polishing lagoon**	**Facultative lagoon**
TSS	30	19.4
VSS	22	16.9
BOD_5	23.6	11
DO	1.73	1.9
Alkalinity	302	231

where: TSS is the total suspended solids; VSS is the volatile suspended substances; TDS is the total dissolved solids; BOD is the biological oxygen demand; DO is the dissolved oxygen.

Table 2. Accumulation of heavy metals by *Anisops sardeus sardeus* from polishing and Facultative lagoons.

Heavy metals	Polishing lagoon (µg/ g dry wt) [a]	Facultative lagoon (µg/g dry wt) [a]
Zn	147.4 ± 1.5	197.1 ± 4.9 [b]
Cd	1.5 ± 0.1	2.7 ± 0.2 [b]
Cu	13.7 ± 0.8	28.2 ± 0.8 [b]
Co	34.4 ± 1.1	37.7 ± 1.7 [c]
Pb	57.8 ± 2.2	59.7 ± 3.6 N.S.
Ni	25.1 ± 1.6	36.9 ± 2.8 [d]

[a] The mean ± SE of 6-7 samples (each sample 0.25-0.5 g dry tissue).

[b] Significantly different from the polishing lagoon, ANOVA with Tukey's test ($p < 0.001$).

[c, d] Significantly different from the polishing lagoon, ANOVA with Tukey's test ($p < 0.139$ and 0.005, respectively).

N.S. Not significant ($P > 0.693$).

Table 3. The mean concentrations of heavy metals in water samples from the *Polishing and facultative lagoons.*

Heavy metals	Polishing lagoon (mg/l)[a]	Facultative lagoon (mg/l)[a]
Zn	12.0 ± 0.4	148.0 ± 0.4 [b]
Cd		3.8 ± 0.1 [b]
Cu	10.3 ± 0.2	27.5 ± 0.2 [b]
Co	9.1 ± 0.8	18.3 ± 0.5 [b]
Pb	41.0 ± 0.4	299.0 ± 0.4 [b]
Ni	86.2 ± 0.2	104.5 ± 1.4 [b]

[a] The mean ± SE of 3 water samples.

[b] Significantly different from the polishing lagoon, ANOVA with Tukey's test ($p < 0.001$).

* Not detected

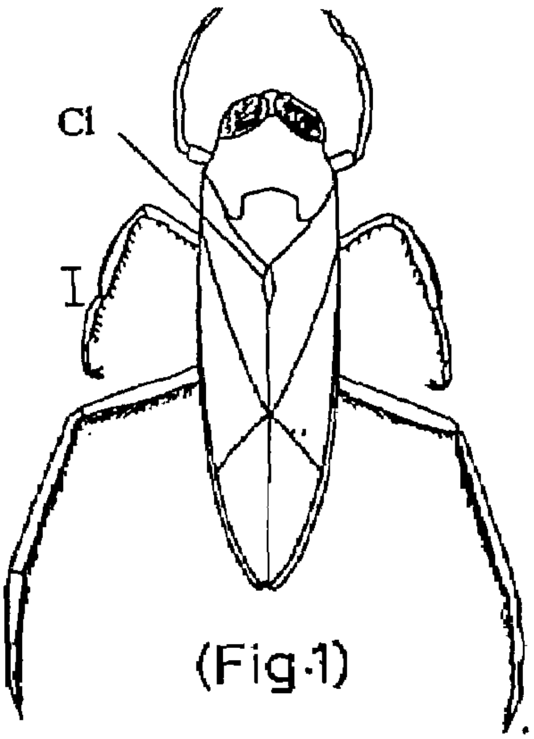
Table 4. Heavy metals concentration in sediments from Polishing and facultative lagoons.

Heavy metals	Polishing lagoon (µg/g)	Facultative lagoon (µg/g)
Zn	201	311
Cd	11	27.5
Cu	22.4	62.6
Co	29	71
Pb	42	56
Ni	39	86

Table 5. The bioaccumulation factor of heavy metals in the tissue of *Anisops sardeus sardeus*

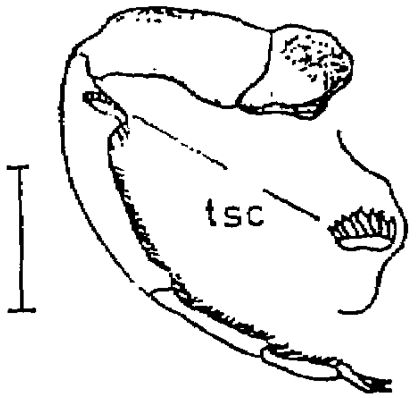
Heavy metals	BAF in polishing lagoon	BAF in facultative lagoon
Zn	12.3	1.3
Cd	1.5	0.7
Cu	1.3	1.0
Co	3.8	2.1
Pb	1.4	0.2
Ni	0.3	0.4

BAF is bioaccumulation factor of heavy metals for each lagoon.

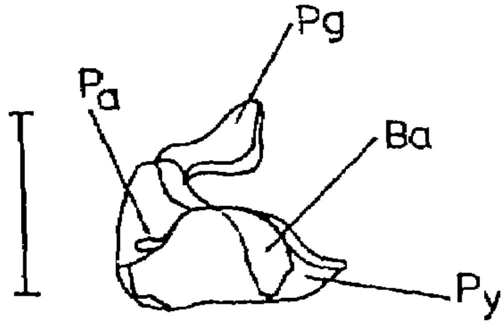


(Fig.1)

(Fig.2)



(Fig .3)



(Fig.4)

Figs. (1-4). ***Anisops sardeus sardeus* Herrich-Schaeffer.** 1 Male, dorsal view, Cl: claval commissure; 2 Lateral view of the head, Pr: prong; 3 Inner surface of male foreleg with enlarged view of tibial stridulatory comb, tsc: tibial stridulatory comb; 4 Male genitalia, Pa: paramere, Pg: proctiger, Ba: basal apparatus, Py: pygophore.