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Significant Effect of Environmental Used Pesticide, Parathion on Transport Properties of Haemocyanin of the Common Shore Crab, *Carcinus maenas*

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ABSTRACT

Pesticides designed specifically for pest, weed control leach into aquatic environments after application, and threaten non-target organisms such as crustaceans that live, spawn and reproduce there. Accumulation of these pollutants in organisms could be detrimental to their physiological properties after exposure. As hemocyanin is pivotal to respiration in crustaceans, the potential ability of pesticides to inactivate or distort hemocyanin would have detrimental consequences. In this study, effects of parathion on the transport properties of the common shore crab, *Carcinus maenas*, was conducted by exposing its hemocyanin to different concentrations (0.5, 10 and 25 mg/ml).The readings on the oxygen properties of the common shore crab were examined at 5 minutes interval in the presence of pesticides. Control experiment had no pesticides. Results showed that parathion significantly altered the hemocyanin oxygen properties ($p<0.05$), which indicates a formation of the covalent bond with the shore crab. There is a need to develop a vision for the future of these unique organisms as well as the communities that depend on them so as to instigate strategies and projects that will enhance the conservation of their biodiversity by using environmental friendly pesticides such as non-chemical pest control and weed control methods may be recommended to reduce adverse effects on *C. maenas* and non-target aquatic biota.

INTRODUCTION

Modern agricultural practices, such as fish farming and crop protection via the use of pesticides such as insecticides, fungicides, herbicides, rodenticides and plant growth regulators are increasing rapidly in response to human population growth in the world (Adeogun *et al.*, 2011). Continuous exposure of chemical weeding, without doubt, has remained very popular in the production of crops, because it reduces cost, labor and it is effective in the agricultural sector(Olabode, Sangodele, & Akinpelu, 2016).The continued increase in human consumption and the need for more efficient agricultural activities, without the doubt will be projected to future increase on the existence of pesticides in the aquatic and terrestrial ecosystem (Elias & Bernot, 2014). Pesticides are classified as either synthetic or natural pollutant of agricultural streams that drift and runoff from different sources after application and are detected in underground, surface and well waters. Once in the aquatic ecosystem, they exert their impact and interfere with the pathological activities and disturb the biochemical and physiological processes of non-target organisms such as alterations on cellular energy allocation, modifications of copper-oxygen site, reduced resistance to infection and respiratory distress. Invertebrates are more vulnerable to these pesticides, because they spawn, reproduce and inhibit these habitats (Prasad, 1995; Sousa & Nogueira, 2001; Byrne *et al.*, 2006; Couillard & Burridge, 2014). Haemocyanin is an oxygen transporter protein in the hemolymph of shore crabs (*Carcinus maenas*). It plays a similar role like the hemoglobin that transports oxygen in the blood of vertebrates. It delivers oxygen from the respiratory organs to the tissues of *C. maenas* (Guo *et al.*, 2012).

Pesticides are classified according to their modes of action by interfering and inhibiting the activities of acetylcholinesterase (AChE) in the nervous system of wildlife, resulting in an accumulation of acetylcholine in the synaptic terminals (Sousa & Nogueira, 2001).Methyl parathion (*O, O*- diethyl-*O*-(4-nitrophenyl) phosphorothioate is an organophosphorus pesticide among the commonly used insecticide that act by inhibiting the activities of AChE and targets the central nervous system of insect pests (Verhaar *et al.*, 2000). In the United Kingdom and Sweden, the use of parathion has been prohibited due to its high toxicity effect to mammals and humans (U.S.D.H.H.S, 2001), though still in use in Greece and Mexico to control a wide range of pest (Mallatou, 2002).Unlike other pesticides, parathion accumulates in the environment for several months unnoticed after application until the effect is observed on the affected non-target organism (U.S.D.H.H.S, 2001; Iyer & Iken, 2013). This type of incident occurred in Canada in Bay of Fundy New Bruns wick and resulted in mortalities of American lobsters (*Homarus americanus*) after application of azamethiphos, a bath treatment used for sea lice control in caged salmon fish in fish farming (Couillard & Burridge, 2014). It is in the light of this, that this investigation was designed to assess the Ecotoxicological effect of parathion on the transport properties of the hemocyanin of the common shore crab, *C. maenas*, the non-target organism of the aquatic biota.

The shore crab, *C. maenas* is native to Europe and northern Africa and known to be among the Portunidae group (swimming crabs) that has been fished commercially for several years (Roman & Palumbi, 2004).*C. maenas* is a functional species for several studies in toxicology (Dissanayake & Bamber, 2010), and has been recommended as an indicator species for the monitoring of pesticides contamination, because these herbicides have been associated with respiratory distress in crabs (Prasad et al. 1995).

MATERIALS AND METHODS

Reagents and test organism

Adult shore crabs were collected and maintained in the aquarium room in biological science department Swansea University the United Kingdom.

Methyl parathion-ethyl, 98.9 % pure, was purchased from Sigma Aldrich Chemical Company, Dorset United Kingdom.

Purification of Haemocyanin

Hemolymph was withdrawn from the pereopods of *C. maenas* using a 27-gauge hypodermic needle. The hemolymph was centrifuged at 2,000 x g for 5 min at 4^0C to separate hemocytes from hemolymph. Extracted hemolymph samples were pre-calibrated in (100 mM Tris, 5 mM each of $MgCl_2$, $CaCl_{2,}$ pH 7.5). The concentration and purity of crustacean hemocyanin were assessed using the standard A280: A350 ratio. This protocol was amended from Coates et al. (2013).

Effect of parathion on the transport properties of hemocyanin of *Carcinus maenas*

Purified hemocyanin containing 0.25 mg/ml was incubated in the presence of increasing concentration of parathion (0.25 to 1 mg/ml) for a period of 5 min prior to absorbance readings using a UV-VIS, 2550 Spectrophotometer. The absorbance readings were indicated across the spectrum from 300 to 500 nm using a quartz cuvette. The maximum peak was determined between 340 nm and 350 nm, indicating oxygenated hemocyanin (Coates. & Nairm, 2014). In the absence of parathion, spectral profiles were used to determine the negative controls. Assays were performed at room temperature using 100 mM sodium phosphate buffer, pH 7.4.

Statistical analysis

All hemocyanin assays were performed in triplicate on three different times. Results are presented as the mean ± standard error (n = 3), and assays were examined using T-test. All analysis was done using Microsoft Excel data Analysis tool Pak, 2007).

RESULTS AND DISCUSSION

Significant effects of parathion on transport properties of hemocyanin of *C. maenas*

The maximum absorption peak of *C. maenas* hemocyanin was observed at 349 nm which is typical for oxygenated hemocyanin and corresponds to the aromatic residues of Cu-O_2-Cu respectively (Figure 1). The bands at 349 nm significantly descended by exposure to parathion at increasing concentrations of 0.25, 0.5, and 1 mg/ml, (Figure 2), indicating a great decline in the degree of oxygenation on the transport properties of hemocyanin of *C. maenas*. This is, therefore, speculated that parathion is able to bind to oxygen binding active site (Cu-O_2-Cu) and form a covalent bond with the oxygen transport properties resulting in potential distress in oxygen supply from the respiratory organs to the tissues of *C. maenas*. Maximum peaks were at 349 nm indicated as the control in the absence of pesticide. Oxygen binding sites of *C. maenas* control assays included 1.0mg hemocyanin, and PBS (pH 7.4) as the buffer in the absence of pesticide and activity measurements were recorded.

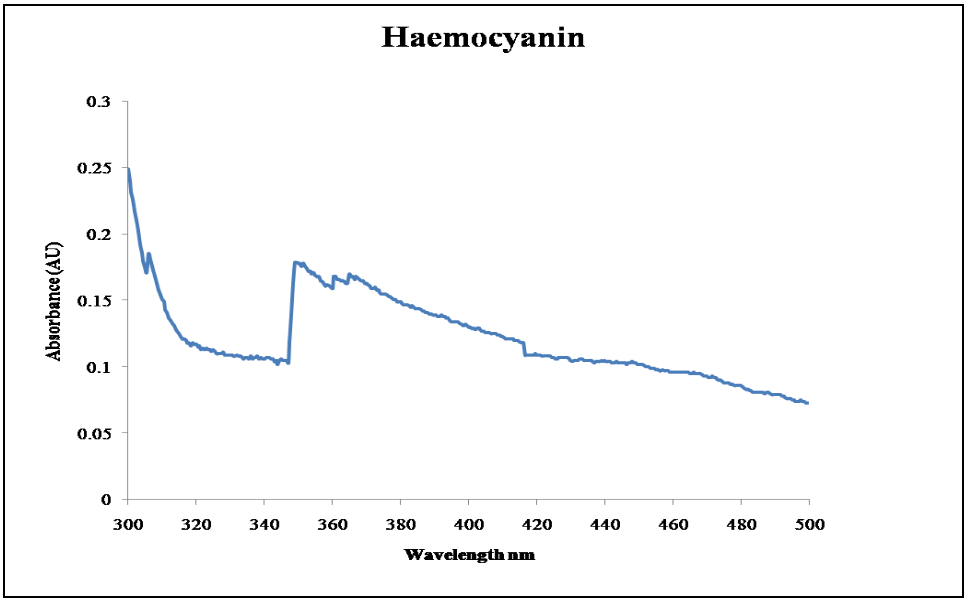

Figure 1: Absorption spectra of total hemocyanin from *Carcinus maenas.*

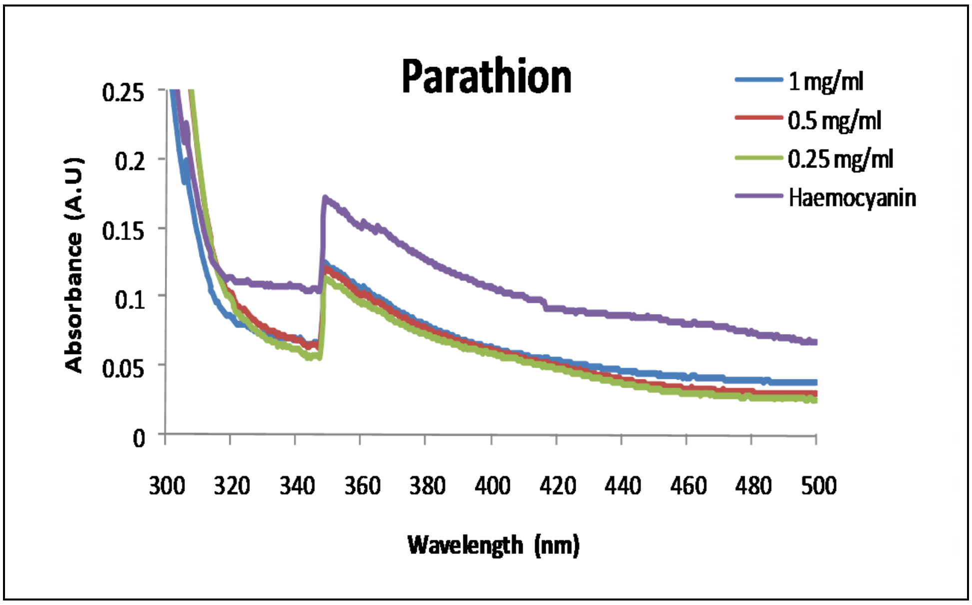

Figure 2: Activity measurement of hemocyanin of *Carcinus maenas* at different concentrations

DISCUSSION AND CONCLUSION

The aim of this research was to investigate the significant effects of parathion on oxygen properties of *C. maenas*. The hemocyanin-oxygen dynamics is a copper that contains pigments in the hemolymph of crustaceans and some mollusks and is essential for their survival. The copper combines with oxygen in the ratio of 1:2 (oxygen: copper: $Cu\text{-}O_2\text{-}Cu$), and gives a copper concentration which equals the measure of hemocyanin content. In the spectroscopic analysis, the bands at 349 nm of *C. maenas* were observably descended, respectively with the addition of parathion. It is detected that parathion directly penetrated the copper oxygen-active sites and interfered with the haemocyanin-oxygen dynamics, thus it is speculated that parathion can bind to the oxygen-binding active site of *C.maenas* in a substrate-like manner, but instead of being oxidated, we suggest that parathion probably alkylates the histidine residue in the active site of *C.maenas* therefore irreversibly inactivating the haemocyanin properties and its affinity which is important for their survival. It is also suggested that existence of respiratory distress as a consequence of parathion toxicity must have occurred after exposure (Srinivas et al, 2013). This further evidences the fact that parathion can bind directly to oxygen binding active site, therefore, form a covalent bond between hemocyanin oxygen dynamics and results in the significant decline of the absorption peak at 349 nm. This result is therefore plausible to a research by Guo *et al.*, (2012) that further demonstrated and indicated a significant effect of copper-oxygen binding site of *Oncomelania hupensis* after exposure to 4-(chloroacetic) catechol. This effect may be stipulated to result in an impairment of oxygen transport, therefore, leading the organism to a progressive hypoxia as observed by (Zou et al, 2006).

This potential effect further indicates that parathion pollution on the environment because of runoff from its source of an application can make *C.maenas* more susceptible to hypoxia and oxygen impairment. This result is required to be used to access the onset duration of the severity of hypoxia and its effect on invertebrates. The implication of this study is that pesticides, such as parathion, designed specifically to kill and control pest can significantly impair hemocyanin oxygen affinity of *C. maenas*, thus leading to alterations in oxygen uptake as indicated by Williams (2012).The toxicity of parathion to other non-target organisms such as fish and invertebrates in the aquatic environment has been observed to cause alterations in physical properties and functions *C. maenas* oxygen-active sites ($Cu-O_2-Cu$). Therefore, it is important to take into consideration the exposure amount of pesticides such as parathion to avoid adverse effects on hemocyanin of non-target organisms such as crustaceans. Additionally, this information may assist to design better environmental friendly pesticides such as non-chemical pest control and weed control methods to decrease potential impacts it may create on non-target biota in the aquatic environment. This could be done by improving new methods specifically to address the unique toxicology effects of these pesticides particularly their impacts on the variety of terrestrial, aquatic and marine organisms.

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Conflict of Interest

Authors have declared that there are no conflicts of interest

Authors Contribution

EO designed and carried out the experiment and wrote the first manuscript. DCG did the statistical analysis and graphics and edited the manuscript; OAA and MOM reviewed and edited and TA proof-read the manuscript

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