ENVIRONMENTAL TOXICANTS AND THEIR EFFECTS ON SPECIES AND ECOSYSTEMS

SUPPLEMENTARY BOOKLET

32nd Annual Sessions of the Institute of Biology Sri Lanka

NATIONAL SCIENCE FOUNDATION

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Contents

Message by the President Institute of Biology, Sri Lanka

One of the greatest challenges of today, both locally and globally, is to combat the problem of environmental contamination, which has caused irreparable damage to our planet and its living beings. Scientists are therefore continually striving to generate up to date information on the status of air, water and soil pollution and the resulting adverse impacts, and to formulate means of restoration and repair. Using this information, governments and other authorities responsible for the maintenance of healthy ecosystems attempt to implement mitigatory measures in the hope of reducing the devastating consequences of these environmental pollutants.

One of the foremost objectives of the Institute of Biology Sri Lanka is to promote the acquisition, dissemination and interchange of biological knowledge that would be useful to policy makers, researchers, academics and the general public. With regard to environmental contamination, such knowledge would relate to the status of pollution of our natural ecosystems, assessing harmful impacts on species and ecosystems, and developing and recommending methodology for environmental restoration. This supplementary booklet titled "Environmental toxicants and their effects on species and ecosystems", which would be circulated at the 32nd Annual Sessions of the Institute of Biology 2012, is a collection of articles written by members of the Institute. The booklet provides some basic information on the status of pollution of wetlands and other aquatic systems, on identified impacts of selected environmental contaminants on species, and on the use of bioindicators, both plant and animal, that could be used to monitor environmental contamination.

We are very grateful to the National Science Foundation (NSF), Sri Lanka, for providing financial assistance for this publication. It is hoped that such information will promote research interest among those who read it.

Mayuri R. Wijesinghe

Impact of soil pesticides on microbial activity of different tea soils in Sri Lanka

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Summary

Use of soil pesticides is an integral component in modern agriculture to ensure high agricultural yield by controlling insect, mite and nematode pests, diseases and weeds. Nevertheless, soil pesticides causes water and soil pollution, affects on non–target organisms in the soils and human health indirectly. Since the effects of soil pesticides on microbial activity in tropical soils have been poorly researched, 10 soil pesticides were tested on different tea soils representing different Agro Ecological Regions (AER), elevations and organic matter status (Deniyaya, Kottawa, Hantana, Passara, Rathnapura and Talawakelle) and rehabilitated soil with Guatemala and Mana and from an undisturbed forest. The soils were exposed to soil fumigants (Dazomet and Metham Sodium) insecticides (Cadusafos and Carbofuran) nematicides (Phenamiphos) and herbicides (2,4-D, Diuron, Glufosinate Ammonium, Glyphosate and Paraquat) at recommended rates. The CO_2 evolution rate was determined using Anderson (1982) method as a measure of change in soil microbial activity due to application of different soil pesticides.

Reduction of soil microbial activity in tea soils due to application of tested pesticides was significant ($P<0.05$) and ranged from 8.2 to 60.1%. Among the soil pesticides tested, Metham Sodium and Glufosinate Ammonium showed greater negative effects. Similar trends were observed with Mana, Guatemala and Forest soils. Compost treated soils resulted in improving microbial

activity in all tea soils by 9.5% and in Mana, Guatemala and Forest soils by 11.7%.

Introduction

The soil health is an important indicator of long term fertility of agricultural soils in any agro ecosystem. Agro chemicals are known to cause negative effects on soil microbial communities while maintenance of soil organic matter through incorporation, mulching, green manuring and minimum use of agro chemicals etc. enhance soil biology (Goyal *et al*. 1999; Wolters 2000).

Tea (*Camellia sinensis* (L.) O. Kuntze) is grown in a range of climates and soils. Hence the tea plant is subjected to various types of insects, nematodes, diseases and weeds. The use of pesticides form an integral component of tea cultivation to ensure high agricultural yield in the integrated management of pests. However, the Tea Research institute of Sri Lanka advocates least priority for pesticide usage and promotes adhering to cultural and biological methods owing to worldwide concerns on health, environment and MRLs. The impact of pesticides on soil, water and environment however, needs to be addressed as it depends on several factors such as properties of pesticides, properties of soils, condition of the sites and management practices (Misra and Mani, 1994). Soil properties affecting the movement of pesticides include soil texture, soil permeability, and organic matter content etc.(http://www.aces.edu/department/crd/publications/ANR-737.html).

Soil microbial activity as a determinant of studying the impact of soil pesticides in different agricultural soils has not been adequately researched. The fate of pesticides used in tea in Sri Lanka (Watawala *et al.* 2005) and their non target effects were identified as important in view of environmental safety and soil biodiversity. The present study evaluated the effect of ten soil pesticides on microbial activity in different tea soils representing different Agro Ecological Regions (AER), elevations and organic matter status.

Methodology

Composite soil samples were collected from different Agro Ecological Regions representing different soil types i.e. Deniyaya (DNY), Kottawa (KTW), Hantana (HNT), Passara (PSR), Rathnapura (RTN) and Talawakelle (TLW) for the study. Soils from rehabilitated lands with Mana (*Cymbopogon confertiflorus*) and Guatemala (*Tripsacum laxum*) and undisturbed forest (FRT) also were collected.

Soil pesticides *viz.* Dazomet, Metham Sodium (Fumigants), Carbofuran, Cadusafos (Insecticide), Phenamiphos (Nematicide), 2,4 – D, Diuron, Glufosinate Ammonium, Glyphosate and Paraquat (Herbicides) at recommended rates given by the Tea Research Institute of Sri Lanka were used with compost and untreated control for comparison. Treated soils were incubated *in vitro* for 2 weeks and exposed for laboratory analysis for determination of microbial activity using Anderson (1982) method.

Results and Discussion

1. Microbial activity of tea soils as affected by different pesticides

Overall, the results revealed a significant ($P<0.05$) effect by the soil pesticides on microbial activity of tea soils of different agro ecological regions. The level of impact on soil microbial activity by the various pesticides varied with the soil type with different soil organic carbon contents and, textures. Pesticides are highly adsorbed by organic matter and clay particles in the soils. Soils with more clay and organic matter tend to hold water and dissolved chemicals longer. Hence, the microbial activity was significantly low in such soils due to negative effects on microorganisms by pesticides persisted longer (Fig.1). TLW with high carbon contents and clayey loamy texture treated with soil pesticides.

Metham Sodium, Dazomet, Carbofuran, Cadusafos, Phenamiphos and Glufosinate Ammonium showed significant effects on microbial biomass in

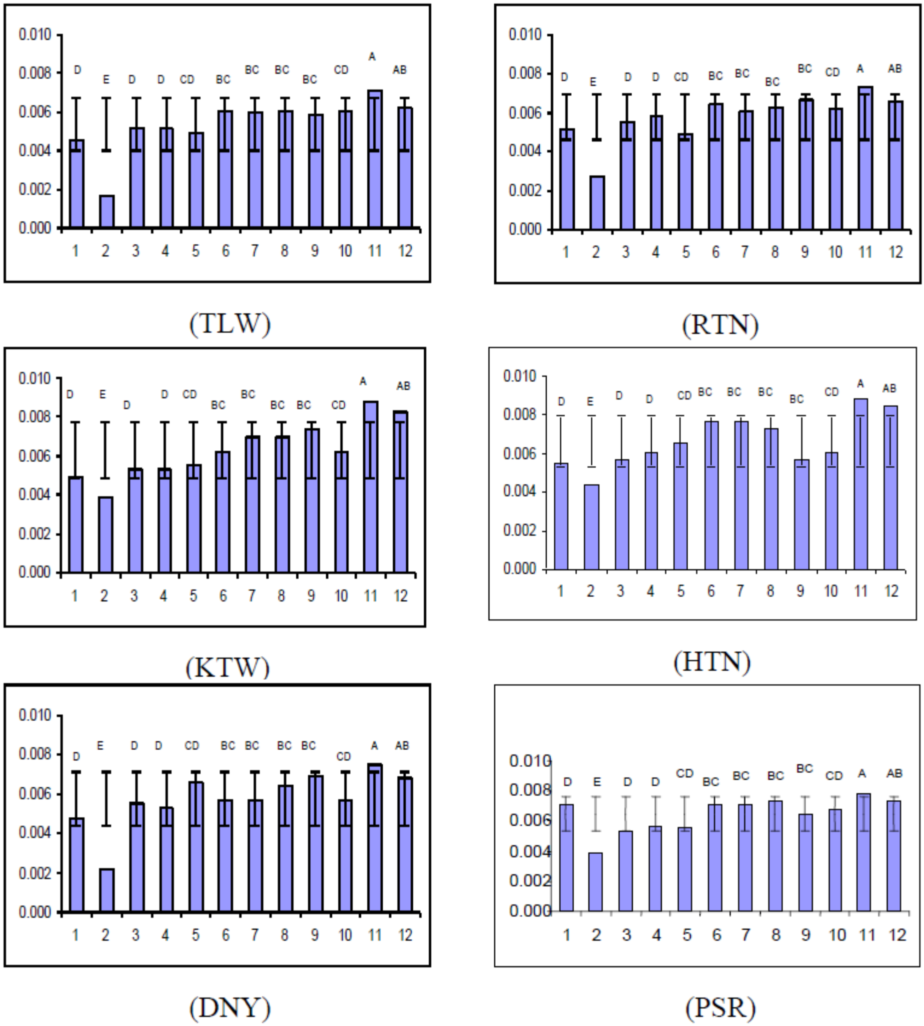


Figure 1. Mean microbial activity of tea soils of different agro ecological regions treated with soil pesticides. (X axis – Soil pesticides : 1- Basamid, 2- Metham Sodium, 3- Carbofuran, 4- Cadusafos, 5- Phenamiphos, 6- 2, 4-D, 7- Diuron, 8- Glufosinate Ammonium, 9- Glyphosate, 10- Paraquat, 11- Compost, 12- Untreated; Y axis – Mean CO_2 evolution rate (g/10 g of soil/day).

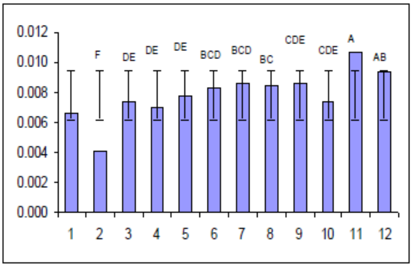
soil while the effects due to Diuron, Glyphosate, 2,4- D and Paraquat was not significant (Fig.1).Contrary, compost treated soil showed the highest microbial activity probably due to higher microbial biomass and considerably high organic matter. Therefore, in situations with lower soil microbial activity in soils affected due to contamination of pesticides, incorporation of compost would rectify the effect as a bioremedial action. Table1 shows the percentage changes in microbial activity of tea soils due to various pesticide applications.

Table 1. Percentage changes in microbial activity in tea soils in agro ecological regions after pesticides application

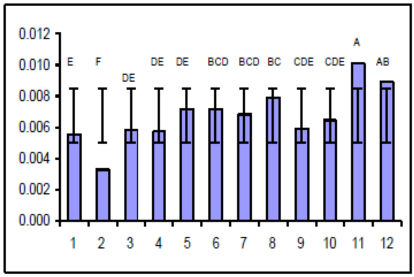
Type of pesticides	Active Ingredient	TLW	KTW	DNY	RTN	HNT	PSR	Mean	SD
Soil Fumigants	Dazomet	-26.5	-40.0	-29.7	-22.2	-34.8	-2.5	-30.6	13.1
	M.Sodium	-73.5	-53.3	-67.6	-58.3	-47.8	-47.5	-60.1	10.7
Insecticides	Carbofuran	-17.6	-35.6	-18.9	-16.7	-32.6	-27.5	-24.3	8.2
Nematicides	Cadusafos	-17.6	-35.6	-21.6	-11.1	-28.3	-22.5	-22.8	8.5
	Phenamiphos	-20.6	-33.3	-2.7	-25.0	-21.7	-25.0	-20.7	10.2
Herbicides	2,4-D	-2.9	-24.4	-16.2	-2.8	-8.7	-2.5	-11.0	9.0
	Diuron	-4.4	-15.6	-16.2	-8.3	-8.7	-2.5	-10.6	5.6
	G Ammonium	-2.9	-15.6	-5.4	-4.2	-13.0	0.0	-8.2	6.1
	Glyphosate	-5.9	-11.1	1.4	1.4	-32.6	-12.5	-9.4	12.6
	Paraquat	-2.9	-24.4	-16.2	-5.6	-28.3	-8.8	-15.5	10.4
Compost		14.7	6.7	10.8	11.1	4.3	6.3	9.5	3.9

2. Microbial activity of forest and rehabilitated soils as affected by different pesticides

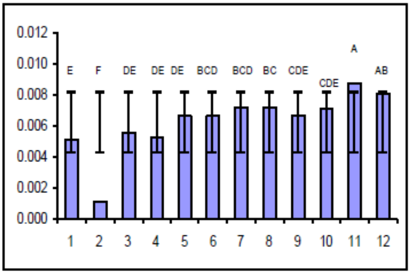
The rehabilitated tea soils with Guatemala (GTM) and Mana (MN) and undisturbed forest soils (FRT) are expected to be comparatively rich in organic carbon and resultantly a greater soil microbial activity. Results indicated a significant effect on such soils by the soil pesticides (Fig. 2). The importance in improving the soil microbial activity through rehabilitation was highlighted in view of bioremediation of contaminated tea lands. Data also revealed an apparent possibility in reaching soil microbial activity of an undisturbed forest.



(GTM)



(MN)



(FRT)

Figure 2: Mean microbial activity of rehabilitated tea and forest soils treated with different pesticides. (X axis – Soil pesticides : 1- Basamid, 2- Metham Sodium, 3- Carbofuran, 4- Cadusafos, 5- Phenamiphos, 6- 2, 4-D, 7- Diuron, 8- Glufosinate Ammonium, 9- Glyphosate, 10- Paraquat, 11- Compost, 12- Untreated; Y axis – Mean CO_2 evolution rate (g/10 g of soil/day)

Table 2 shows the percentage changes in microbial activity after soil pesticides application in rehabilitated and forest soils. Metham Sodium treated soil showed significantly lowest microbial activity while compost treated soil showed the highest.

Table 2: Percentage changes in microbial activity in rehabilitated and forest soils after soil pesticide application

Type of pesticides	Active Ingredient	FRT	MN	GTM	Mean	SD
Soil Fumigants	Dazomet	-38.1	-36.4	-29.4	-34.6	4.6
	M. Sodium	-62.9	-86.4	-55.9	-68.4	16
Insecticides	Carbofuran	-35.1	-31.8	-21.6	-29.5	7
Nematicides	Cadusafos	-36.1	-34.1	-25.5	-31.9	5.6
	Phenamiphos	-19.6	-18.2	-17.6	-18.5	1
Herbicides	2,4-D	-19.6	-18.2	-11.8	-16.5	4.2
	Diuron	-23.7	-11.4	-7.8	-14.3	8.3
	G. Ammonium	-11.3	-11.4	-9.8	-10.8	0.9
	Glyphosate	-34	-18.2	-7.8	-20	13.2
	Paraquat	-27.8	-12.5	-21.6	-20.6	7.7

Conclusion

The recommendations on soil pesticides by the Tea Research Institute of Sri Lanka are fair in view of pesticidal efficacy and residual effects on the final product. However, the non target effects of such soil pesticides irrespective of the active ingredient on soil microbes in tea soils of different agro ecological regions in Sri Lanka was clearly demonstrated in this study. The fate of different soil pesticides in different tea soils with varying levels of organic matter, soil textures and pesticide adsorption capacities was evident. Observations were similar to findings of Goyal *et al*. (1999) and Misra and Mani (1994). Hence, a location specific and more rational pesticide recommendation over the existing blanket recommendation based on soil type was made clear in this study as a precision agricultural practice with concerns on leaching and retention capacity of pesticides and effects on soil health.

Also, the bioremedial properties by important Good Agricultural Practices (GAPs) such as soil rehabilitation with Guatemala or Mana and incorporation of organic amendments in rectifying affected tea lands were shown. Adherence to such cultural practices shall accomplish improving soil microbial activity and overall soil productivity and also reaching biological status of an undisturbed forests.

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Effects of aquatic pollutants on fish and amphibians

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Introduction

Contaminants in freshwater ecosystems, of mounting concern today, include a diversity of substances ranging from agrochemicals to pharmaceutical compounds and heavy metals. These contaminants enter aquatic systems directly from applications or through discharge into the water bodies or in runoff from contaminated fields. In some cases they may also enter water bodies as wind-borne drift, in particular when agrochemicals are sprayed to nearby fields. Aquatic organisms therefore are often exposed to repeated influxes of these pollutants in varying concentrations. In the case of fish and amphibians, these contaminants enter their bodies via the oral pathway as a result of consumption of contaminated plants and animals, or due to absorption through their skin. Additionally, in fish and in newly hatched tadpoles with external gills, pollutants may also be absorbed through the gills. Once a contaminant is within the organism it has the potential to induce both direct and indirect adverse effects upon it.

Ecotoxicologists have, over the years, attempted to predict the effects of environmental contaminants on different aquatic species. Nevertheless, due to confounding effects and the inability to provide controlled environments in the field, toxicity of substances are most commonly assessed through laboratory trials. From among the different aquatic taxa used for toxicity tests, fish and amphibians have been used more regularly as model taxa; the reasons being that fish and amphibians, during both the larval and adult stages, are convenient to rear as they are relatively tolerant of captive conditions, and are easier to handle. Additionally, they could easily be observed and hence monitored. Tadpoles are particularly useful for assessing changes in growth and development since this occurs over a comparatively short period of time.

Some recorded impacts of pollutants on fish and amphibians

Before moving on to some of the observed impacts of aquatic pollutants, I will briefly explain some of the basic terms used in ecotoxicology. For exposures of fish and amphibians, the amount of the contaminant an individual is exposed to, at one time or over a given time period, is described as the 'concentration'. This is more relevant in an aquatic environment than the term 'dose', which is normally used when a substance is administered or injected. 'Toxicity' is used simply to reveal the degree of damage induced by a particular contaminant in the exposed organism. Some contaminants are toxic in any quantity, some even in trace amounts, and others only beyond a certain exposure level. The terms 'acute' and 'chronic' are used to describe both the patterns of exposure and the nature of damage. Consequently, an acute exposure is when organisms are exposed to a single large concentration of a contaminant, while a chronic exposure is when organisms are repeatedly exposed to low concentrations. Similarly, damage is acute if effects induced are direct and sometimes lethal or has the potential to inflict grave damage in the exposed organism, but chronic if the damage induced is sublethal and long term.

Let us now briefly review some of the recorded lethal and sublethal impacts of environmental contaminants on fish and amphibians.

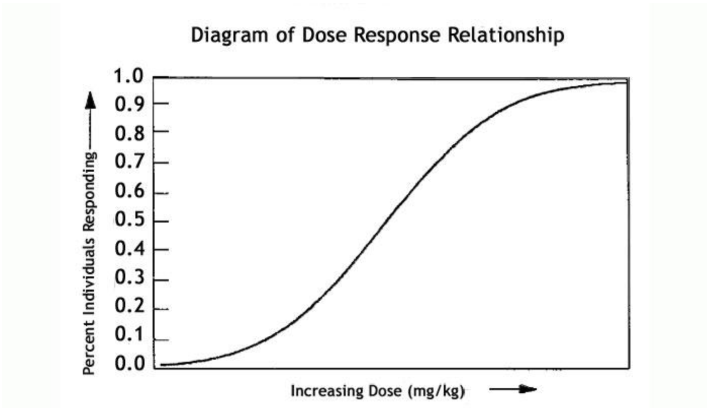
Effects on direct mortality

Without doubt the most detrimental impact a contaminant can have on exposed organisms is to cause direct death. Not surprisingly, scores of studies have demonstrated the potential of various substances to enhance the levels of direct morality in fish and amphibians. Let me cite a few examples from studies done in Sri Lanka. De Mel and Pathiratne (2005), through their trials, predict that exposure to either 7.85 mg l^{-1} of carbaryl or 0.60 mg l^{-1} of carbosulfan can result in 50 % mortality of fry in the common carp (*Cyprinus carpio*). These are the LC50 values (concentrations causing 50 % mortality) of the two substances. In other studies, Ranatunga *et al.* (2011) report that the Mozambique Tilapia (*Oreochromis mossambicus*) suffered relatively high levels of mortality (around 20 %) at ecologically relevant levels of cadmium, while Ranatunga *et al.* (2012) records similarly high mortality levels in tadpoles exposed to the same heavy metal. Mortality is generally expressed as a dose-response curve, which is a graphical representation of the relationship between the quantity of the contaminant to which the organism is exposed and the magnitude of the induced impact. Dose-response curves are typically sigmoidal (Fig. 1a & b), but under laboratory conditions, where only a limited number of concentrations are tested, we usually obtain a linear relationship. In some instances, however, we may get a bell-shaped curve as shown in Fig. 1c. This is when mortality at the higher concentrations is lower than that observed at the lower levels of exposure, a pattern known as hormesis (Calabrese and Baldwin 2002). It is reported that such patterns are more frequently observed when organisms are exposed to endocrine disruptors (Cavieres *et al*. 2002), although the mechanisms behind it are yet not properly understood.

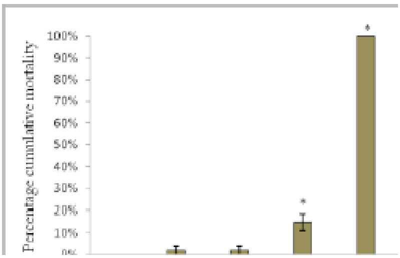
Ideally a range-finding test should be conducted to identify levels that would cause intermediate levels of mortality. However, in instances when one is interested in ascertaining impacts of field levels, then such range-finding tests are not strictly required.

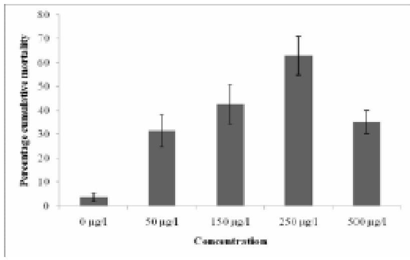
Sublethal impacts

In addition to causing direct mortality, most contaminants have the potential to manifest a range of sublethal impacts in the exposed organisms. Among the most commonly observed sublethal impacts in fish and amphibians are growth retardation and delays in development.



(a)





(b) (c)

Figure 1: Dose-response curves (a) Typical sigmoidal curve (b) curve for fry exposed to cadmium and (c) curve for tadpoles exposed to carbofuran (hormetic response). (Sources: Ranatunga *et al.* 2011 and Jayatillake *et al*. 2011)

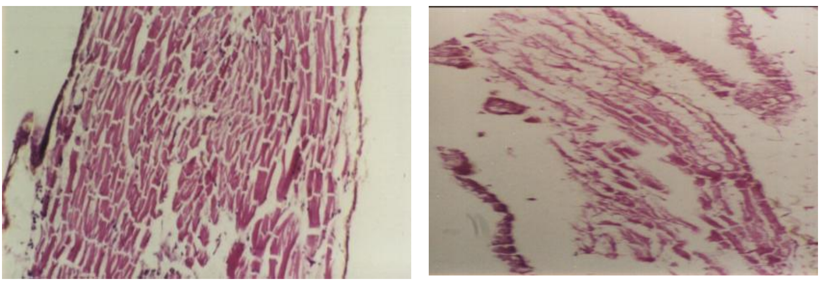
A case in point would be the study by Witeska *et al.* (2010) where they demonstrate that eggs and fry of the barbel (*Barbus barbus*) exposed to 100 μgl^{-1} of copper or cadmium markedly reduced larval growth, with differences between controls and exposed groups being more pronounced in the case of cadmium. For amphibians, a developmental delay during the larval stage would generally result in a corresponding delay in metamorphisis. For instance, it has been demonstrated that the organophosphates diazinon and chlorpyrifos both reduce rates of metamorphosis in tadpoles of the Asian common toad *Duttaphrynus melanostictus* (Sumanadasa *et al.* 2008; Wijesinghe *et al.* 2011). In both fish and frogs growth and developmental delays may be due to reduced swimming which in turn results in lower rates of feeding and hence lower nutrition, or because energy that could otherwise be devoted to growth and development is used up for maintenance mechanisms to deal with xenobiotic stressors (Rowe *et al.* 1998).

Some contaminants have shown the ability to disrupt normal behavioral patterns. For example, impairment of the predator-avoidance behavior has been observed in the tadpoles of the gray tree frog (*Hyla versicolor*) when exposed to carbaryl (Bridges 1999). Similarly, the ability to recognize alarm signals and predators were reduced when juvenile chinook salmon (*Oncorhynchus tschawytscha*) was exposed to an organophosphate pesticide. Alterations in swimming intensity of fish fry exposed to Cd, and tadpoles exposed to diazinon, have been demonstrated by Ranatunga *et al*. (2012) and Sumanadasa *et al. (*2008), respectively.

There have been several observations in recent years of the incidence of deformities in frogs markedly increasing, giving rise to the speculation that pesticides are one of the major contributory factors in causing these abnormalities. Evidence for this is found in a study by Jayawardena *et al.* (2011) where the loss of limbs occurred in *D. melanostictus* tadpoles when exposed to four pesticides during the embryo stage. Another example would be the observed abnormalities in the tadpoles of the same species when exposed to carbofuran (Jayatilleke *et al.* 2011).

Those described so far are some of the more apparent and easily observable types of damage inflicted by environmental contaminants in fish and amphibians. Additionally, there are some effects induced by contaminants that may not be clearly visible externally. These include alterations in tissue structures, hematological parameters and hormonal levels. I will briefly describe evidence with respect to both histology and hematology as the last mentioned will be dealt with in another paper presented at this workshop.

Environmental contaminants are capable of inducing lethal or sublethal impacts through effects on critical organs/tissues such as the kidney, liver, gills and muscles. For instance, chlorpyrifos was seen to cause significant damage to the gill and muscle tissues of *D. melanostictus.* The gills of the exposed larvae showed architectural distortion with markedly reduced primary and secondary gill lamellae while the tail muscle suffered severe atrophy and disintegration (Bandara *et al.* 2008; Jayatilleke *et al.* 2011) (see Fig. 2). The hematological profile of a species is a good indicator of the levels of environmental stress and, consequently, changes in blood parameters have been used to assess effects of environmental pollution (Vinodhini and Narayanan 2009). Some of the parameters that have shown sensitivity to contaminants are RBC and WBC levels, clotting times, pack cell volume (PCV) and hemoglobin content. One of the outcomes of such changes is immune-suppression, which in turn leads to increased susceptibility to common diseases, as has been noted in certain species.



(a) **(b)**

Figure 2. Histopathological damage caused by carbofuran in tadpoles of D. melanostictus (a) normal tail muscles and (b) degenerated tail muscles. Source: Jayatillake et al. (2011).

It should also be kept in mind that the damage may vary depending on the nature of the contaminant, frequency and duration of exposure, characteristics of the exposed individual and the tolerance limits of the study species. Under field conditions impacts could be influenced by a multitude of environmental factors such as solar radiation, water quality parameters, and sediment characteristics.

Far reaching consequences

It is easy to envisage how damage induced at the level of the individual organism can have far reaching consequences. Direct death or sometimes indirect mortality brought about by the inflicted sublethal impacts will inevitably result in population declines. Apart from reduced survival, it is documented that when growth is retarded and the resulting individuals are smaller in size than the norm, these individuals will have a reduced ability to obtain food resources, be more prone to attacks by predators (Semlitsch *et al.* 1988), have a lower a reproductive potential or suffer from reproductive failure (Marian *et al.* 1983), once again leading to enhanced mortality and lower recruitment. The subtle changes such as the alterations in blood parameters and tissue structure will also progressively lead to the malfunctioning of the affected tissues and organs and affect normal physiological processes ultimately contributing to the occurrence of unhealthy individuals with reduced rates of survival. A substantial reduction in populations may possibly, in the long term, result in the disappearance of small localized populations with consequential implications that would be manifested at the higher trophic levels.

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Impacts of heavy metal pollution on wetlands

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Heavy metals – what are they?

Heavy metals are natural constituents of the earth's crust. Although there is no clear definition of what a heavy metal is, density is regarded as the defining factor. Heavy metals are thus commonly defined as those having a specific density of more than 5 g/cm^3 (Diaz *et al*. 1998). Many of these elements are essential to the life of plants and animals in very low concentrations. Some of them are volatile and some become attached to fine particles that can be widely transported to faraway places. Thus, production and disposal of heavy metals are local, environmental distribution is global (Adams *et al*. 2010).

Indiscriminate human activities have drastically changed the biochemical cycles and balance of some heavy metals on earth. Between 1850 and 1990, production of copper, lead and zinc had increased 10-fold mainly due to industrial use and automobiles (Benson *et al*. 2007).

Any metal or metalloid may be considered a “contaminant” if it occurs in places where it is unwanted and in unwanted concentrations, or in a form or concentration that is a detrimental to the environment, biota or to humans (Reena *et al*. 2011).

How do heavy metals reach wetlands?

In tropical countries water is no doubt a common natural resource and so are the wetlands. Wetlands are among world’s most complex and productive ecosystems with characteristic water, soil and biodiversity components. For centuries, wetlands have been a convenient dumping ground for waste generated on land. Thus these ecosystems are increasingly been contaminated with various heavy metals through emissions from the rapidly expanding industrial and commercial activities , disposal of metal wastes, leaded gasoline and paints, fertilizers, sewage sludge, pesticides, spillage of petrochemicals, and atmospheric deposition.

Heavy metals that have entered wetland ecosystems may experience three immediate pathways of transport and translocation as shown in Fig. 1.

Figure 1. The fate of heavy metals when entered into a wetland

The risk – PBT effect

Once the heavy metals have entered the wetland, they endure a long time.

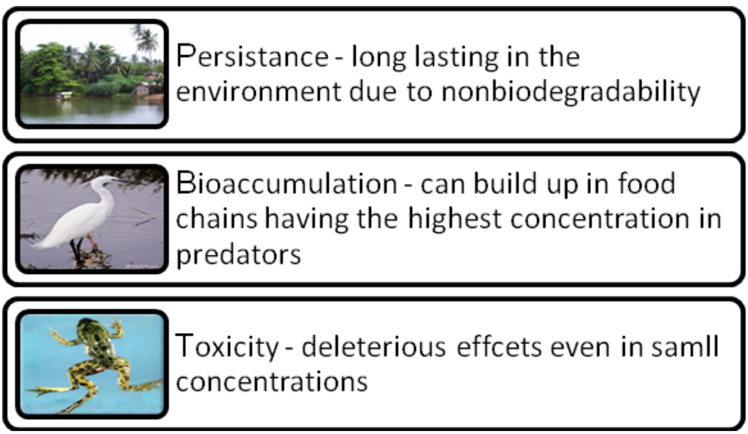

Figure 2. PBT risk of heavy metals

Most metals bioaccumulate to some degree in the tissues of aquatic organisms (Karadede and Unlu 2010). However, the extent of bioaccumulation varies. Some species accumulate metals to high levels (e.g. zooplankton), while other species such as fish closely regulate internal concentrations or sequester the metal with cellular binding proteins (e.g. metallothioneins). Generally, bioconcentration is the most important route of uptake for most metals in water. Only for a couple of metals (mercury, selenium) has it is shown that the food component is more important than the water component (Clearwater *et al*. 2002).

Biomagnification : Some metals are known to biomagnify (Pempkowiak *et al*. 1999). The best recorded example is mercury (Hg), which biomagnifies to a great extent, but only when present in the lipophilic organic form, methyl mercury (MeHg). Methyl mercury is readily bioaccumulated by algal species and subsequently biomagnified through trophic levels and highest in the top level.

The pathway to tissues

The aquatic organism take heavy metals through three ways: First two are from the body itself i.e. through the body surface and the gills and the third one is from the food they consume (Yilmaz *et al*. 2007).

Uptake of heavy metals depends on two factors physiochemical and biological.

1. *Physiochemical factors*

Temperature and pH: Generally, increasing of pH increases the amount of copper hydroxides which are toxic to aquatic organisms. Higher water temperature and lower pH during summer increased the rates of trace metals uptake by the most common fish species *Oreochromis niloticus* (Shakweer and Abbas 2005).

2. Biological factors

Different biological factors are known to influence on the uptake of heavy metals.

Age of an organism : This a major role in metabolism. The age of an organism can increase or reduce the uptake of heavy metals. Cadmium and mercury accumulation have reported to increase with age. In contrast, Mn, Cr, Cu and Zn accumulation decrease with age.

Specific specific effects: Interspecies differences on the accumulation of metals in fish tissue has been demonstrated in laboratory studies. The heavy metal concentrations in tissues of the benthic fishes were higher than in pelagic fishes, due to the variations in their ecology and behavior.

Specific organs : The bioaccumulation of heavy metals usually vary between the organs (skin, gills, liver, muscle, bone, brain and muscle). In general, liver, kidney and gills accumulate more heavy metals due to their higher metabolic rates. Muscle where the metabolic activity is relatively low accumulate less level of metals.

Sri Lankan studies

Being a tropical island, wetlands are one of the most abundant ecosystems in Sri Lanka. Most of these wetlands, especially ones that are situated in urban areas receive a considerable load of pollutants including heavy metals. To mention a few, in a study carried out in Kotte Kolonnawa wetland, it was found that Pb, Mn, Cd, Cr and Cu existed in concentrations which exceeded threshold limits for aquatic organisms (Hettiarachchi 2010). Senartane and Pathiratne (2007), report studies carried out in Bellanwila Attidiya wetland and Bolgoda Lake where water is contaminated with lead, cadmium, copper and zinc and their accumulation in fish tissues.

Although many prior studies worldwide have clearly documented various biological, ecological and behavioural impacts of heavy metals on aquatic life, very little is known about Sri Lankan biota. Here we report some field and laboratory studies of heavy metal induced ecoimmunomodulation in frogs *Euphlyctis haxadactylus (*Indian Green Frog*)* in Bellanwila Attidiya Sanctuary, a polluted wetland, in the Colombo District. Ecoimmunomodulation could be explained as the change in immune system function due to effects of chemical pollutants such as heavy metals. In one study, heavy metals copper, zinc, lead and, cadmium have found to exist in wetland waters in considerable concentrations and these were attributed to cause general immunosuppression in frogs (Madushani *et al*. 2010). In another study carried out in the same natural setup indicated that heavy metals affect immunological parameters that include total white blood cell (WBC) counts, differential WBC counts (Fig. 3), spleen weight/body weight ratio and neutrophil / lymphocyte ratio (Priyadharshani *et al*. 2011 a).

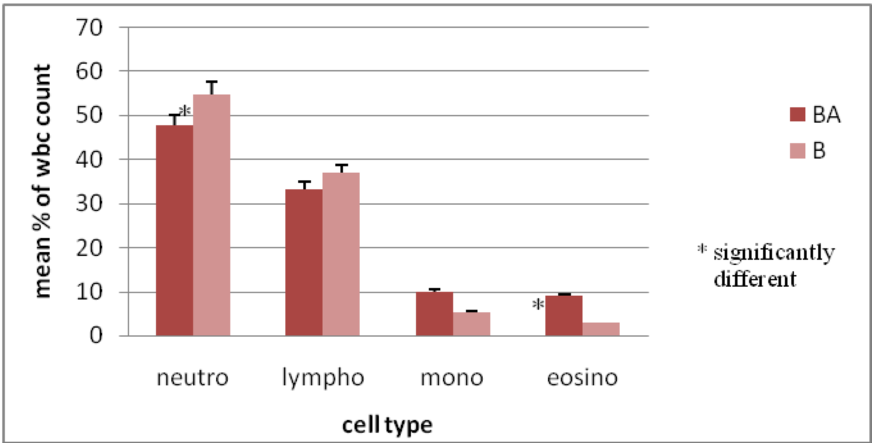

Figure 3. WBC differential counts (neutrophils, lymphocytes, monocytes and eosinophils) in frogs in the polluted (BA) and reference (B) sites

In a similar manner, a laboratory study reports heavy metals induced immunomodulation of phagocytes of *E. hexadactyla* (Fig. 4). Here, all phagocytes showed immune suppression (reduces the activation or efficacy of the immune system) with the increase concentrations of heavy metals, except the blood leukocytes (Priyadharshani *et al.* 2011b).

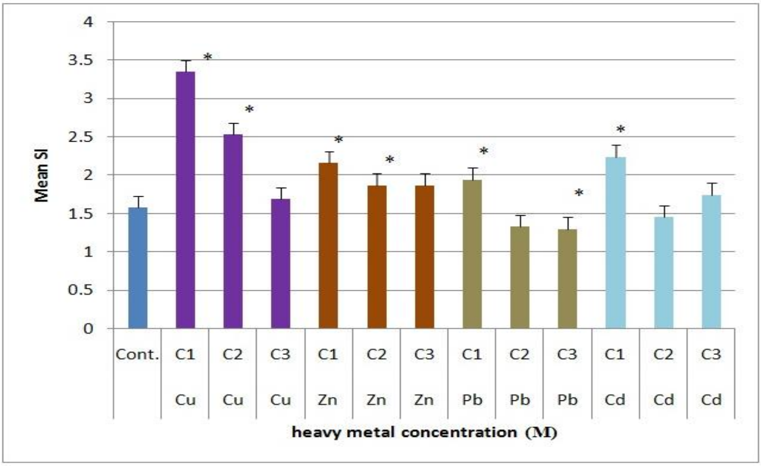

Figure 4. Dose response relationships of *E. hexadactylus* blood leukocytes for selected metal ions. *p<0.05; univariate analysis of variance. Cont - Control C1- 10^{-8} M C2- 10^{-6} M C3- 10^{-3} M.

These results clearly indicate that the heavy metal contamination definitely affects the life of the amphibians. It is realistic to assume that this condition will have impacts of other aquatic taxa in different degrees.

Concluding remarks

Heavy metals are ubiquitous in our environment, and exposure to them are practically inevitable. There are adequate evidences in the literature to show that heavy metal toxicity creates profound impacts on different systems and processes in aquatic animals globally. For far too long, researches in Sri Lanka have paid scant attention to the problem of heavy metal pollution in wetlands. Only scientifically and politically integrated efforts could guarantee that wetlands which are regarded as “environmental kidneys” in purifying water, are safe from heavy metal contamination.

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Marine environmental toxins: an increasing threat to coastal waters

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Introduction

Ocean is the life support system of the earth. Healthy and functioning oceans provide essential services to mankind. The crossroad has now been reached where the cumulative effects of what is taken from and what is put into the world's oceans have reduced substantially and the ability of marine ecosystems to produce desired and needed economic and ecological goods and services. If present trends are allowed to continue and if there is continuing failure to responsibly manage the oceans and coastal regions, there will be much greater losses in the near future - at a much accelerated pace. It is clearly evident that what we once considered to be inexhaustible and resilient is, in fact, finite and fragile. Increasing amounts of pollutants will result in the deterioration of water quality which will adversely affect the long-term health of marine life and marine ecosystems, the quality of seafood and, ultimately, affect human health. For example, heavy metals such as mercury can now be found in high concentrations, not only in benthic organisms but also in pelagic fish such as tuna or swordfish. Unprecedented amounts of toxins and high concentrations of harmful chemicals released into the air and into coastal waters present the greatest threat to human health. Untreated or poorly treated sewage sludge and municipal waste discharged into coastal regions can introduce pathogens, heavy metals and a variety of toxins which can affect the food chains of benthic and pelagic life. The consumption of such sea products can have long-term deleterious effects on human health.

Harmful algal blooms and marine bio-invasions

The global scale movement of ships has resulted in a massive transfer of freshwater and marine organisms to the surrounding waters through ballast water and biofouling. Ballast water is a major source of biological invasions around the globe offering a conducive situation for bacteria, viruses, algae, dinoflagellates and a variety of macro-faunal larval and cyst stages to flourish. Although all the world oceans are linked, many species function in localized ecosystems that have evolved natural controls. Health hazards are generally caused by build-up of toxins in the food chain by Harmful Algal Blooms (HAB). The toxic effects of these harmful algae can lead to fatality in human beings through paralytic shellfish poisoning (PSP) and diarrhoeatic shellfish poisoning (DSP).

As an example, there are over 18 species of animals and plants documented along the Indian coasts that might have invaded and then established. They can cause deleterious effects to local flora and fauna through their toxigenic, proliferative and over-competitive characteristics. Black-stripped mussel, *Mytilopsis sallei*, has been reported from Mumbai and Visakhapatnam. This species is a native of tropical and subtropical Atlantic waters and is reported to have invaded the Indian waters sometime during 1960s (Rao 2005). Green crab, *Carcinus meanas*, a native of Europe, is found in Sri Lankan waters. The molluscs and crustacean population on which this crab preys upon can, therefore, be affected (Anil *et al*. 2002). *Vibrio cholerae* is the causative bacteria for the human epidemic cholera, and can be transported through ballast water. As the bacteria are capable of forming associations with plankton, their survival and sojourn in the ballast water tanks are much easier.

Studies have recorded 26 new zooplankton and dinoflagellate species from the inner harbor area that were not recorded in the literature. Among these two species found in ship's ballast water namely *Microsetella* sp and *Prorocentrum* sp (Ranatunga and Siyambalapitiya 2010). *Ceratium Furca,* *Ceratium fusus, Peridinium* sp., *Protoperidinium grande, Protoperidinium obtusum,*and *Protoperidinium robustum* were the six species of potentially harmful red tide forming dinoflagllates which were recorded from coastal waters adjacent to Colombo port (Senanayaka and Ranatunga 2010). A baseline biological survey in Hambantota port has revealed the presence of species such as *Ceratium furca, Chaetoceros* sp., *Thalassiosira* sp., *Rhizosolenia* sp. and *Protoperidinium* sp. that are known for red-tide formation (Wijetunga and Ranatunga 2012).

This form of impact of shipping has resulted in international conventions for the control and management of ships' ballast water and sediments. Yet to enter into force, it envisages the introduction of mandatory ballast water management from 2009, but no later than 2016, in order to eliminate the common practice of vessels loading and discharging untreated ballast water. In order to formulate and implement laws and regulations with regard to the ballast water exchange, monitoring of plankton species in and around ports of Sri Lanka is essential. Sri Lanka's strategic location within close proximity to the east-west maritime rout ensures more than 4500 bulk carriers, cargo vessels and oil tankers arrive to Colombo harbor annually. With the recent port development projects, vessels arriving in Sri Lanka will be increased by several orders of magnitude, increasing the threat of bio-invasions. Preventing the introduction of ballast and wastewaters or excessive amounts of nutrients, helps avert harmful algae blooms, preserves the quality of sea water and of sea products and, most importantly, eliminates the threats to human life and sustaining the ecological balance of coastal regions.

Sewage and waste dumping in the ocean

Sewage has been identified as the top priority issue globally transferring pathogenic microbes (UNEP 2002). Pathogens can survive in seawater from a few hours to many weeks (Table 1). Sewage can be carried by run-off, rivers, floods, tides, groundwater and storm surges. Urbanization and population growth increasingly leading to disposal of sewage in concentrations far above the assimilative capacity of the receiving environments, with little scope for natural attenuation across the land and rivers before wastes find their way to the coast.

Table 1. Survival times of major pathogens and indicators of sewage in the marine environment (Source: Ashbolt 1995)

	Group name	*Survival time*
Virus	Adenovirus	50 days
	Echovirus	2 days - 46 weeks
	Hepatitis A	> 24 days
	Poliovirus	2 - 130 days
	Reovirus	> 4 days
	Rotovirus	2 - 34 days
Bacteria	*Escherichia coli*	-
	Faecal coliforms	2 hours - 2 days
	Fae. Streptococci	2 hours - 12 days
	Salmonella spp.	12 hours - 5 days
	Shigella spp.	< 15 - > 70 days
	Vibrio spp. Days	indigenous / < 6
Protozoa	*Entamoeba histolytica*	Unknown
	Giardia intestinalis	unknown

Although sewage treatment is seen as a way to reduce nutrient loads before release to the environment, this depends on the use of high levels of processing which can be costly, require large amounts of wetland areas or both. Many industries and vessels continue to pour raw sewerage, noxious chemicals, toxic and radioactive wastes directly into the sea. For centuries, man has over-fished a bountiful region, driving some species to the brink of extinction, all the while spewing filth into those very waters that sustain us. Deteriorating water quality can be reversed through stricter and enforceable regulatory controls of industrial effluent discharged into the coastal environments. Unfortunately, there are no national standards hitherto for dumping waste to the marine environment.

Seafood poisoning

Scombroid fish poisoning

Histamine may develop in a variety of fish species as they decompose. Eg: tuna, marlin, sardines, anchovy, herring and mackerel. Ingestion of histamine may cause scombroid poisoning in humans, which may lead to a variety of symptoms, including rashes, nausea, vomiting, diarrhea, hypotension, palpitations and muscle weakness (Emborg *et al*. 2005). Paralysis and death have also been reported in cases of scombroid poisoning. Because of its potential for human illness, the U.S. Food and Drug Administration (FDA) has set 50 ppm as the upper limit for histamine in domestic and imported fish.

Ciguatera Poisoning

The ciguatera chain starts when herbivorous animals consume some dinoflagellates and their toxins. These toxins concentrate and transform in their tissues, and pass them up the food chain, further accumulating and concentrating in each step. Some jacks, snappers, and groupers are known to cause ciguatera poisoning in the tropics. In the Indian Ocean, ciguatera commonly occurs in Madagascar, Mauritius and the Seychelles and has also been reported from Sri Lanka (Rey 2012). Ciguatoxic seafood imported from these regions can cause poisoning in any area of the world.

Oil and chemical spills

Oil spills include releases of crude oil from tankers, offshore platforms, drilling rigs and wells, as well as spills of refined petroleum products (such as gasoline, diesel) and their by-products, heavier fuels used by large ships such as bunker fuel, or the spill of any oily substance refuse or waste oil. Oil penetrates into the structure of the plumage of birds, reducing its insulating ability, thus making the birds more vulnerable to temperature fluctuations and much less buoyant in the water. Most birds affected by an oil spill die unless there is human intervention. Marine mammals exposed to oil spills are affected similarly. Oil coats the fur of sea otters and seals, reducing its insulation abilities and leading to body temperature fluctuations and hypothermia. Ingestion of the oil causes dehydration and impaired digestions. Because oil floats on top of water, less sunlight penetrates into the water, limiting the photosynthesis of marine plants and phytoplankton. This, as well as decreasing the fauna populations, affects the food chain in the ecosystem.

The risk of a major oil spill around Sri Lanka is getting worse over time with accelerated international surface vessel trafficking. The potential eco-toxicological impact of oils spills on Sri Lankan marine environment is relatively unknown. Many institutes and industries have stored a large amount of dispersants to be used in an accidental oil spill. However, such dispersants have not been tested on marine organisms. Therefore, predicting responses to oil spills that would minimize the eco-toxicological impacts have been very difficult.

There are a few examples of accidental oil and chemical spills in Sri Lanka. In 1999, the ship M V Melishka ran aground at a distance of about 1000 m off the Bundala coast. Reports indicate that the 200 mt of heavy fuel oil and 16500mt of fertilizer released caused damage to the marine environment. SS Thermopylae Sierra that had been anchored in the sea off Panadura for four years, has recently sunk. . The ship has left 20 to 30 tonnes of fuel which poses an environmental threat to the coastline. MT Granba tanker was sunken off Trincomalee in 2009 with 6250 mt of sulfuric acid in a location nearly 53 nautical miles offshore and at a depth exceeding 3500 meters. However, there are no studies on the impacts of such incidents.

Although it is too early to predict any impact due to offshore oil exploration in the Gulf of Mannar, it is imperative to closely monitor the activities as there are many ecologically important marine ecosystems in close proximity to it. Impacts will be very crucial if the exploration is developed into a production level.

Heavy metals in the marine environment

Heavy metals are among the most toxic, ubiquitous, and persistent contaminants in coastal and estuarine habitats. Metals that are dissolved or suspended in the water column can degrade seawater quality and can become available to floating and swimming organisms and bottom-dwelling filter feeders. Although, the majority of environmental metals reside in bottom sediments, the principal transport pathway is in the water column. Unfortunately, almost nothing is known about the physical and chemical pathways of these toxicants in our marine environment. Worse still, for coastal habitats there has been no reliable data on even the concentration of trace metals.

Organic pollution

Persistent organic pollutants (POPs) are toxic chemicals that adversely affect human health and the environment around the world. Because they can be transported by wind and water, most POPs generated in one country can and do affect people and wildlife far from where they are used and released. They persist for long periods of time in the environment and can accumulate and pass from one species to the next through the food chain. Many of these chemicals proved beneficial in pest and disease control, crop production, and industry. These same chemicals, however, have had unforeseen effects on human health and the environment. POPs include a range of substances that include:

- Intentionally produced chemicals such as polychlorinated biphenyls (PCBs) and DDT. PCBs are used in electrical transformers and large capacitors as hydraulic and heat exchange fluids, and as additives to paints and lubricants. DDT is still used to control mosquitoes that carry malaria in some parts of the world.

- Unintentionally produced chemicals, such as dioxins, that result from some industrial processes and from combustion (for example, municipal and medical waste incineration and backyard burning of trash).

Studies have linked POPs exposures to diseases, or abnormalities in a number of wildlife species, including fish, birds, and mammals. Impact to wildlife sound an early warning for people. In people, reproductive, developmental, behavioral, neurologic, endocrine, and immunologic adverse health effects have been linked to POPs. People are mainly exposed to POPs through contaminated foods. Less common exposure routes include drinking contaminated water and direct contact with the chemicals. In people and other mammals alike, POPs can be transferred through the placenta and breast milk to developing offspring. However, readily available literature on levels of organic pollutants and their toxic effects on aquatic life is extremely scarce in Sri Lanka.

Marine debris

Marine debris are any persistent, manufactured or processed solid material discarded, disposed or abandoned in the marine and coastal environment. Debris inflicts major impacts on the health of the ocean as a whole as well as on humans and wildlife. Sharp items like broken glass or metal cans cut beachgoers, while disposable diapers, condoms, and old chemical drums introduce bacteria, toxic compounds, and other contaminants into the water. Marine wildlife suffers from dangerous encounters with marine debris as well, facing sickness and death from entanglement or ingestion of man-made objects. And the pervasive problem of marine litter even impacts economic health. Debris that is dumped into the ocean can remain in the ocean from a few weeks to several hundred years (Table 2).

Table 2. Marine Debris Degradation Timeline

Item	Years
Paper Products	2-6 weeks
Plastic 6 pack beverage holder	6 months
Agriculture Products	2-6 weeks
Waxed Milk Carton	3 months
Plastic Bag	10-20 years
Cotton Rope	3-14 months
Wool Socks	1-5 years
Plastic Film Canister	20-30 years
Plywood	1-3 years
Rubber Boot Soles	50-80 years
Foam Plastic Fishing Bobbers	80 years
Cigarette Butts	1-5 years
Leather	50 years
Disposable Diaper	80 years
Tin Cans	50 years
Foam Plastic Cups	50 years
Aluminum Cans	80-200 years
Plastic Beverage Bottles	450 years
Monofilament Fishing Line	600 years

Source: www.oceanconservency.org

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Air pollution monitoring using lichens as indicators

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Introduction

Air pollution is the contamination of air by discharge of harmful substances from various sources. These are primary pollutants and include mainly sulphur dioxide, nitrogen dioxide, smoke and suspended particulate matter (SPM), which may react with each other or by radiation and ambient temperature, to produce secondary pollutants. Example: primary pollutants such as nitrogen dioxide and sulphur dioxide can be converted to secondary pollutants such as ozone and peroxy acetyl nitrate respectively. Until recently air pollution problems have been local and minor because the atmosphere has the ability to absorb and purify minor quantities of pollutants that contaminate it. Industrialization, introduction of motorized vehicle, excessive use of fossil fuels and population explosion are the major factors that contribute towards the growing air pollution issues. During the last century, several major air pollution episodes had been reported from various industrial cities, in USA, UK, Europe, Australia and Japan.

The atmospheric pollutants, which have the greatest potential for affecting growth of crop plants and trees are oxides of sulphur, nitrogen and photochemical oxidants such as ozone and peroxy acetyl nitrate. These are formed by burning of fossil fuels in industries, electric plants and vehicles. All the plants are not equally sensitive to pollutants; some species may be very sensitive to a particular pollutant, while another species may be resistant. In some instances air pollutants increase the susceptibility of plants to diseases, parasites and various environmental stresses such as drought (Awang 1995).

The specific sensitivity of plants to various air pollutants has resulted in the use of plants as indicators to detect and monitor air pollution (Burton 1986). The nature of symptom may vary according plant species and type, concentration and period of exposure to a particular air pollutant. However, the responses elicited by the plants represent the end result of the integration of influences of several factors. Therefore, it may not provide specific information on the concentration of pollutants in the environment when compared to physicochemical measurements using sophisticated equipment (Awang 1995). Nevertheless, indicator plants are inexpensive substitutes for long term continuous monitoring air pollutants.

Lichens are unique group of symbiotic organisms, formed due to intimate association between two different plants, an alga (algae) and a fungus. They grow attached to trees, rocks or man-made substrates in three different growth forms, foliose, fruiticose and foliose. The plant body is referred to as the thallus and unlike other plants, lichens are devoid of roots and leaves. The unique anatomy of the thallus, specially the absence of cuticle makes them easily affected by external harsh conditions. Lichens have long been shown to be very sensitive to air pollution and have been used as bioindicator since the 19th century. Finnish naturalist, William Nylander observed that lichens had nearly disappeared from Luxembourg Garden of Paris, France (Hawksworth 2002). From numerous field observations and from long-term monitoring surveys of the lichen communities throughout Europe, the potential of lichens to be used as biomonitors have been well explained (Seaward 1993; Nimis *et al.* 2002).

Although the lichen flora of Sri Lanka has been studied in the past by several world renowned lichenologists (Leighton 1870, Nylander 1990; Kurokawa & Mineta 1973; Hale 1980, 1981; Awasthi 1991) none of these include any reports related to air pollution. Further, very little or no systematic studies have been done in tropics to find the effect of air pollutants on lichens. As such, the work described herein is the pioneer attempt made to investigate the potential of lichens in air pollution monitoring in tropical milieu. With no previous knowledge related to diversity and distribution of lichens in an area, it is rather difficult to assess the effect of air pollution on lichens. Therefore, in this study, a method successfully employed in Europe, to monitor air pollution using lichens was introduced to examine the effectiveness of tropical lichens as indicators of air pollution.

During the last two decade the quality of air in Colombo metropolitan area has found to be gradually degrading mainly due to the increase in vehicular emissions. Thus the aims of this work were to: (1) study the epiphytic lichen vegetation of the humid tropical environment; (2) develop a method to monitor air pollution using lichens; (3) establish a baseline of lichen vegetation for long-term monitoring survey in the area.

Methodology

For this study thirty-one sites falling on six transactions (A, B, C, D, E, F) diverging from Colombo city to suburbs were selected Length of each transect was 50 km and separated by an angle of 30^o. Five sites (Ai, Aii, Aiii, Aiv, Av) were located on each transect and the distance between two sites on a transect was 10 Km and area of each site was $1Km^2$. The site in Colombo city (CC) was a common site for all transects (Fig.1). The survey area is situated in the Western Province where there is no significant variation in the climatic conditions among the sites. The method adopted is largely based on the German guidelines with few modifications with respect to the selection of sampling trees (VDI 1995).

Eight trees in each site, 3 from *C. nucifera*, 3 from *M. Indica* and 2 from *A . heterophyllus* were randomly selected for the study. For the registration, area of tree trunk between 0.5 m from the base to 1.5 m height was selected as the sampling area. Coverage and frequency of lichens were recorded by placing $250cm^2$ (25cm X 10cm) quadrate randomly at four places on the bole of each tree (VDI 1995). All lichen species found within the gird, number of individuals of each species and the number of grid units in which a particular species recorded were estimated. Lichens were identified according to their morphology, reproductive structures and chemistry of the thalli.

At each site the bark pH of the trees, their exposure to light, and relative humidity values were measured. The study sites were categorized into four classes, according to traffic (very high, high, moderate and low) and land use (industrial, urban, suburban, village), which were used as categorical variables in the analyses.

Lichen diversity of each site was determined using Shannon's diversity index (Batten 1976). $\boldsymbol{H' = -\sum p_i (\log p_i)}$ Where, H' = Diversity p_i = the proportional abundance of the ith species. According to the lichen diversity index values obtained for different study sites, they were categorized into four lichen diversity classes, Low, moderate, high and very high.

Index by atmospheric purity, which is a diversity measurement that developed to quantifying environmental conditions, primarily air pollution using lichens as bioindicators (De Sloover and Le Blanc 1968) was calculated for each site using frequency or percentage cover of lichens and a factor of tolerance of toxicity given by ecological index (i.e. the average number of species, which coexisted with each species (De Sloover and Le Blanc 1970).

For calculation of IAP index the following equation was used. (De Sloover and Le Blanc 1970).

$$IAP = \sum_{1}^{n} (Q \times f) / 10$$

Where n = Number of species recorded

Q = Ecological index (i.e. the average number of species, which coexisted with each species)

f = Cover or frequency of each species

Data were analysed using SPSS PC-10 statistical package.

Results

A total of 47 lichen genera were recorded in all the study sites and commonly represented families were Arthorniaceae, Graphidaceae, Pyrenulaceae, Physciaceae, Trypetheliaceae, Lecanoraceae, Tricotheliaceae and Thelotremataceae. The commonest genera were *Arthonia, Arthothelium, Arthopyrenia, Graphis, Graphina, Sarcographa, Bacidia, Lecanora, Pyrenula, Anthrocothecium, Buellia, Dirinaria, Pyxine, Trypethelium, Thelotrema.* Majority of lichens in the study sites were crustose type. Foliose and fruiticose types only found in sites in sub-urban and village sites and they were less prominent. Since there is no previous information on the epiphytic lichen flora of the area, changes that have occurred in lichen communities cannot be assessed.

The mean difference of lichen diversity indices of land use 1 (industrial) significantly differs from land use 3 (suburban) and 4 (village) ($P \leq 0.005$).The mean difference between land use 1 (industrial) and 2 (urban) was not significantly different. Land use 2 (urban) was significantly different from land use 3 & 4. Land use 3 & 4 were also found to be significantly different from each other with a P value of ≤ 0.05.

There was a significant difference in lichen diversity between the traffic density 1 (high traffic) and other traffic density classes ($P \leq .0.05$) However, there was no significant mean difference between each of the other traffic density classes.

Correlation coefficient between lichen diversity and bark pH of the three tree species were not significantly different.

The IAP values for each site, the total mean IAP value, standard deviation and coefficient of variance are given in Table 1. The lowest IAP was recorded only once and that was in the city site (CC). The mean IAP value for the study area is 69.2 ± 23.8 indicating 31 sampling sites display high variation of air pollution.

IAP values obtained for different sites along all transects showed a similar trend, Sites in urban and industrial areas gave low IAP values where as the sites in village areas away from city center showed higher values.

Discussion

In general, the crustose lichens are least sensitive to air pollution, foliose lichens are intermediate and fruticose lichens are most sensitive (De Sloover & Le Blanc 1968; Wetmore 1981).On all transects, the sites close to the Colombo city, which is highly polluted due to vehicle emissions, had only crustose lichen species occurring more frequently, while foliose lichen species (*Coccocarpia* sp and *Parmotrema* sp. *Phyllopsora* sp and *Leptogium* spp.) were recorded at a lower frequency in some suburban sites and more frequently in village sites. Fruticose lichen *Roccella* was recorded rarely in sites of the coastal transects away from the city.

A gradual increase in lichen diversity index values observed when moving away from the city center (CC) site to village sites on most transects, suggest that air pollution arising due to vehicular emission could be the main cause of this difference in lichen diversity index. The amount SO_2 and NO_2 level recorder during the same time in the same sites (details not given) showed significantly higher values in the city center site when compared to other sites. These findings are similar to observation made throughout Europe, where decrease in epiphytic lichen floras have been explained mainly by high SO_2 levels before 1970 (Skye 1968 ; Hawksworth and Rose 1970) and more recently correlated with increase in nitrogen pollution (Van Dobben and De Bakker 1996).

High IAP values in sites away from the city and low IAP values in sites closer to the city on all transects suggest that IAP values indicate the air quality of the area and hence it could be regarded as a good method to monitor air pollution (Attnayaka and Wijeyaratne). This high level of air pollution in the city is mainly due emissions from the transport sector. In 2001, at the time this research was conducted the total vehicle fleet in Sri Lanka was around 1,800,000 and about 10% of that would have been in Colombo city and suburbs causing much pollution (Fig. 1). Significant difference in lichen diversity observed between high traffic density and other traffic density classes also endorses the validity of IAP method in monitoring air pollution in tropics.

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Table 1. IAP values for sampled sites, mean IAP value, Standard deviation and lichen diversity values for each site.

Site code	IAP	Diversity index	Site code	IAP	
CC –City site	24	0.8373			
A i	38	0.8805	D i	42	0.9560
A ii	32	0.7026	D ii	66	1.1517
		1.0552			1.4447
A iii	38		D iii	84	3
A iv	49	1.1166	D iv	85	0.9678
A v	62	1.0777	D v	102	1.1495
B i	47	1.0996	E i	43	0.8807
B ii	56	1.0264	E ii	84	0.9117
B iii	51	1.0741	E iii	87	0.8497
B iv	89	1.0825	E iv	93	1.0700
B v	67	1.0526	E v	81	1.2098
C i	43	0.9979	F i	48	0.4626
C ii	81	1.0378	F ii	85	1.0487
C iii	91	1.1378	F iii	87	1.1191
C iv	94	0.9877	F iv	88	0.904o
C v	105	1.2216	F v	101	1.1138
Mean= 69.2			SD=23.8		

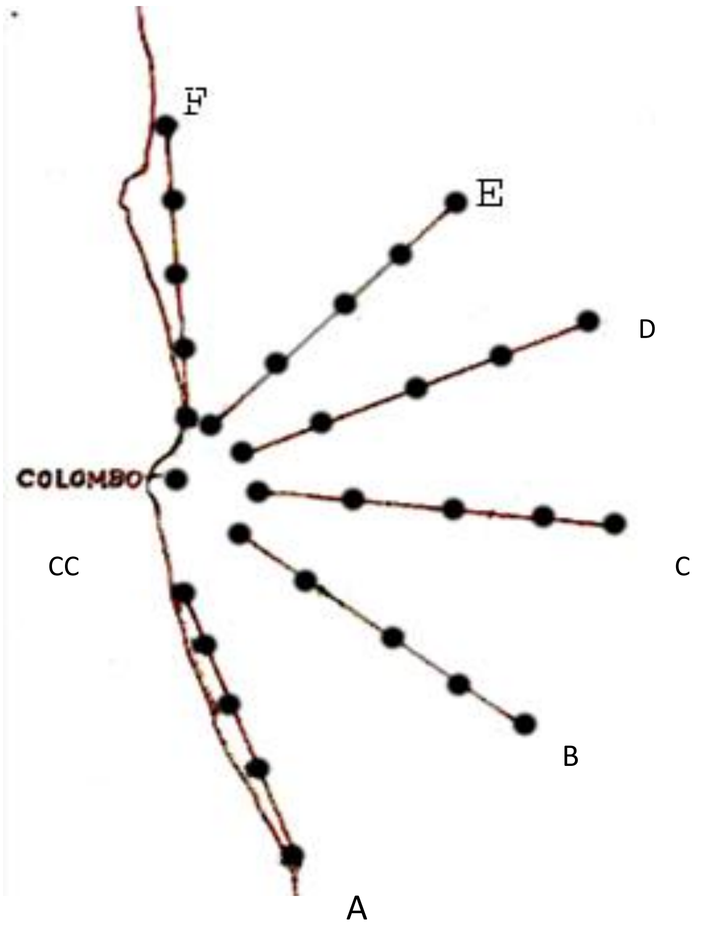

Figure 1. Schematic map of the surveyed area showing six transects and 31 sites

Crustose

Graphis scripta

Lecanora sp.

Foliose

Pseudocyphellaria sp.

Parmotrema sp.

Fruticose

Roccella sp.

Figure 2. Different morphological forms of lichens

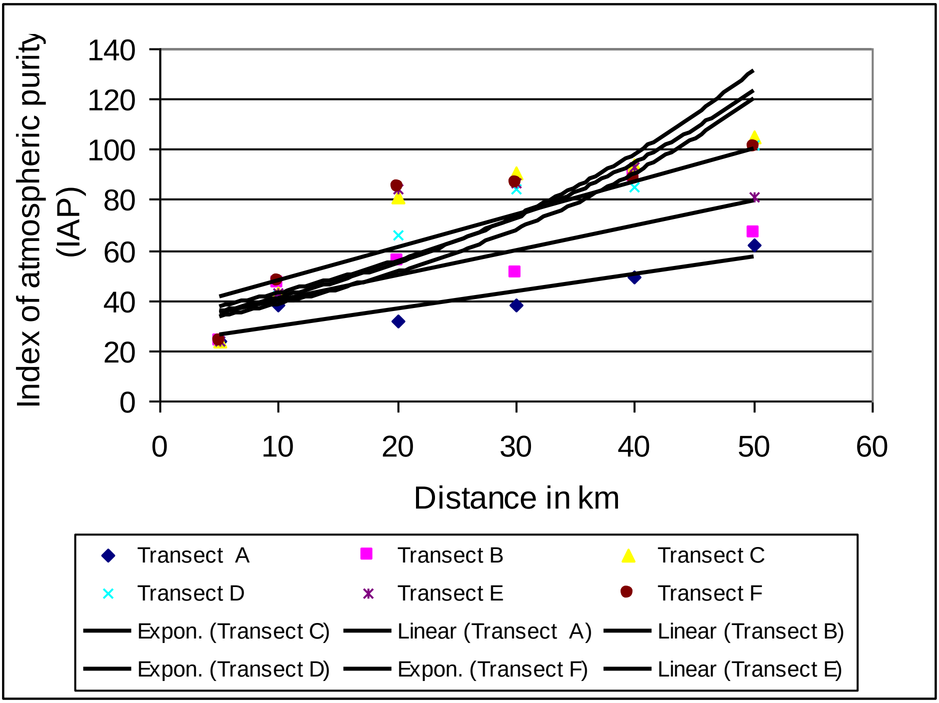

Figure 3. Changes of IAP values along different transects

Tiger Beetles as Appropriate Bioindicators of Environmental Change and Pollution

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What is a "Biological Indicator"?

A biological indicator can be described as "a species or group of species whose characteristics (eg. presence or absence, population density, dispersion, reproductive success) represents the impact of environmental change on a habitat, community or ecosystem, or is indicative of the diversity of a subset of taxa, or of the whole diversity within an area" (Hodkinson and Jackson 2005; Koivula 2011). Bioindicators are a good way to monitor the effects of toxic materials on organisms when this might be difficult to assess through direct toxicity level assessment in nature (Avgin and Luff 2010). A good bioindicator should have a well-known taxonomy and ecology, be distributed over a broad geographic area, have a high degree of ecological specialization as specialized species are far more vulnerable to environmental perturbations compared to generalists, be cost-effective and relatively easy to survey (Rainio and Niemela 2003; Brischoux *et al.* 2009).

Indicator Taxa

Previously, estimations of environmental diversity and quality relied primarily on vertebrates such as large mammals and birds (Regulska 2011). Sea snakes have also been used to estimate biodiversity of coral reefs in the Pacific Ocean (Brischoux *et al.* 2009). In recent years more attention has been directed to studies including smaller organisms such as invertebrates, and earthworm species, molluscs, crustaceans, spiders and aquatic and terrestrial insects are commonly used as bioindicators (Andersen 1997; Bhattachergee 2008; Regulska 2011).

Beetles as Bioindicators

Amongst the invertebrates ground beetles or carabids are frequently used in bioindication and several studies suggest that they are excellent indicators of forest fragmentation, ecological effects of industrial emission, effects of military tanks, agricultural chemicals and heavy metals Rainio and Niemela 2003; Koivula 2011; Regulska 2011).

Heavy metals such as copper alters the locomotor behavior of *Poecilus cupreus* and zinc has a negative effect on reproduction of the same species (Avgin and Luff 2010). Fe is known to accumulate in large quantities in contrast to other heavy metals (Butovsky 2011). However, beetles are known to be weak accumulators of heavy metals in comparison to other arthropod groups but good indicators of the presence of cadmium in the environment (Lukan 2009). Beetles have a high potential as biological indicators in forest and agricultural areas and are used for monitoring pollution from oil, sulfur, herbicides, carbondioxide, insecticides and radioactive phosphorus (da Rocha *et al.* 2010).

Tiger Beetles as Bioindicators

Tiger beetles of family Cicindelidae are worldwide in distribution except in Antarctica, the Arctic above 65° latitude, Tasmania and some oceanic islands like Hawaii and Maldives (Pearson 1988). Further, their systematic and ecology are well known due to the dedication and cooperation of insect collectors and taxonomists (Pearson and Cassola 2005; Putchkov and Cassola 2005). Species are highly habitat specific and each species rarely occurs in more than one or a very few habitat types (Adis *et al.* 1998; Cardoso and Vogler 2005; Satoh and Hori 2005; Rafi *et al.* 2010). They are relatively easy to observe and manipulate, and students of tiger beetles can quite easily census an area during the season of adult activity and reliably find most of the species within a short time (Pearson and Cassola 2005; Pearson 2011). Therefore, tiger beetles have been identified as appropriate bioindicators for inventory studies and monitoring studies and has been used as a valuable tool in conservation research (Rodriguez *et al.* 1998). Species have been used to monitor habitat degradation in forests of Venezuela (Rodriguez *et al.* 1998); predict species richness of butterfly species in North America (Carroll and Pearson 1998); butterfly and bird species in Australia and bird species in the Indian subcontinent (Pearson & Carroll 1998); ecosystem health of Shivalik Landscape, India (Uniyal *et al.* 2007).

Are tiger beetles of Sri Lanka pertinent bio-indicators for monitoring the environment?

Fifity-nine (59) species of tiger beetles are recorded from Sri Lanka, of which 39 are endemic (Dangalle *et al.* 2012a). Records of their taxonomy and distribution are found since 1860 and a substantial amount of literature is readily available (Tennent 1860; Horn 1904; Fowler 1912; Wiesner 1975; Naviaux 1984; Acciavatti & Pearson 1989). Tiger beetles of Sri Lanka are highly habitat specific and restricted to a few habitat types such as river banks, banks of reservoirs, coastal areas and urban habitats (Dangalle *et al.* 2012b). Further, they are easy to observe in nature and can be handled without difficulty. Therefore, the tiger beetles of Sri Lanka are highly suitable to be used as bioindicators, and their relationship with habitat degradation and diversity of other taxa should be studied.

The tiger beetles of Sri Lanka are associated with the aquatic habitats of the country that are rich in biodiversity. The endemic species, *Cylindera* (*Ifasina*) *waterhousei* is found on the banks of Kuru River, Bopath Ella, Ratnapura (Dangalle *et al.* 2011a), and *Cylindera* (*Ifasina*) *willeyi* at Maha Oya, Dehi Owita and a stream at Handapangoda (Dangalle *et al.* 2011b). Four species are associated with eleven (11) reservoir systems that are mostly located in the North-Central and North-Western provinces of the country (Dangalle *et al.*2012a). Pesticides that are commonly misused in Sri Lanka are known to contaminate these water bodies through runoff and seepage (Somasiri 2007). Reservoir habitats are polluted by bathing, washing and livestock watering and cleaning (Somasiri 2007). Kelani river, where *Cylindera* (*Ifasina*) *labioaenea* was recorded (Dangalle *et al.* 2012b) is considered as the most polluted river in Sri Lanka due to many industries discharging both treated and untreated industrial effluents (Ileperuma 2000). These effluents are high in pathogenic bacteria, organic content and heavy metals that may cause gastric cancers and kidney diseases after long term accumulation (Ileperuma 2000). Reservoirs such as the Bomuruella Reservoir in Nuwara Eliya and Kandy Lake in Kandy are also polluted due to industrial effluents and toxic substances (Jayasinghe *et al.* 2011; Yatigammana 2011). Further, coastal habitats of Sri Lanka are damaged due to discharge of raw or poorly treated sewage and industrial effluents, and lagoons near urban areas are degraded by sewage, garbage

Cylindera (Ifasina) waterhousei

Cylindera (Ifasina) willeyi

and waste fuels (Lowry and Wickremeratne 1988). According to Ileperuma (2000) the beach sand at Hendala contain radioactive minerals. Three species of tiger beetles, *Hypaetha biramosa*, *Lophyra* (*Lophyra*) *catena*, *Myriochila* (*Monelica*) *fastidiosa*, are associated with the coastal areas and beaches of Sri Lanka (Dangalle *et al.* 2012b). Therefore, there is a very broad possibility of using the tiger beetles of Sri Lanka as bioindicators to monitor habitat health of aquatic ecosystems of the island. However, more studies have to be done to evaluate the relationship between tiger beetles and habitat, and tiger beetles and other taxa. No two species can precisely reflect each other, and one must be prepared for uncertainty and error when using an indicator. However, as humans will continue to utilize the environment and as biology will continue to compete with economies and social policies, detecting sites and taxa that are threatened and of high conservation value will be essential and biological indicators such as the tiger beetles will be of utmost importance.

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