Studies on components of Blood and its functions

Chapter 1

Impact of organophosphates and other pesticides on blood parameters of fishes and earthworms

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Abstract

The earthworms and fishes are regarded as most agro-economically valuable organisms in India and around the world. The earth worms living underground can make the soil fertile by two means. First, it can flip the down soil upwards and secondly it can make the soil rich with nitrogen produced from waste products. In India and in other parts of the world, both fresh water and marine fishes are consumed largely. An indiscriminate use of pesticides in agriculture may stall the earthworm population and production of fishes by creating different physiological anomalies either by themselves or by their derivatives, though both of the organisms are non-target to the pesticides. Blood performs almost all important and vital physiological functions due to its circulatory nature. It transports necessary nutrients, gases and waste products throughout the body. Therefore, the present study was designed to explore the impact of various pesticides in modulations of hematological/cellular/metabolic and free radical parameters in blood of earthworms and fishes. We observed significant variations in different hematological parameters of these two organisms upon acute exposure to few organophosphates and other pesticides. These results suggest that the acute exposure of these pesticides can affect the non-target organisms and change the balance of eco-system adversely.

1. Introduction:

The earth is diversified with numerous organisms in terms of prokaryotes and eukaryotes and is thus classified under five different kingdoms with a critical consideration regarding their cellular configuration, morphology, anatomy, histology, cytology and different minute variations. The eukaryotic animals are differentiated as vertebrates having the vertebral column and the invertebrates which are devoid of it. The earthworms and fishes, the representatives of invertebrate and vertebrate groups, respectively are regarded as most important organisms in agro-economic system around the world. However, the reasons of their importance are totally different.

2. The Role of Earthworms in Agro-Economic System

Earthworms are the invaders of the earth below ground and are abundant in number particularly in the tropical and temperate zones of the world[1] with more than 3500 species [2, 3]. This annelid can perform multiple functions including making the soil more fertile mainly by two means. First, it can flip the down soil upwards and secondly it can make the soil rich with nitrogen produced from waste products. Earthworms affect the supply of nutrients largely through their burrowing activities as they produce aggregates and pores (i.e., biostructures) in the soil and/or on the soil surface, thus affecting its physical properties, nutrient cycling, and plant growth [4, 5]. The biogenic structures constitute assemblages of organo-mineral aggregates. Their stability and concentration impact soil's physical properties and SOM (Soil Organic Materials) dynamics. Besides they also affect some important ecological processes which influence their "functional domain" [6, 7]. These concentrate nutrients and resources are further exploited by soil microorgansms [8, 9]. The gut contents are often enriched in organic matter, nutrients, and water compared with bulk soil due to selective foraging and can thus foster high levels of microbial activity [10, 11]. Therefore, this organism is regarded as the "Friend of Farmers" particularly in India.

3. The Role of Fishes in Agro-Economic System

Biologically, fishes are the most abundant vertebrate class with more than 32,500 species diversified into fresh water, estuarine and marine [12]. It is well known fact that the fishes are one of the richest sources of Polyunsaturated fatty acids (PUFA) [13], easily digestible proteins [14], different vitamins and minerals [both macro- and micro-minerals, 15]. The amount of minerals is even richer in the estuarine and marine fishes than those of fresh water fishes [16]. Apart from the nutritive values, fishes are the sources of different derived materials like liver oils, ointments rich in vitamins particularly vitamin D and other products rich in minerals. The fish scales have been used as raw materials for artificial pearls and decorative beads. A fish substance known as isinglass became an important raw material for glue production because of their gelatinous consistency. As the fish offal and heads or bones trimmings are

rich in protein their use have gone beyond culinary purposes due to the fact that different fish types contained different levels of nutrients. Therefore, they could be used as fish meal or converted into fish silage. Fish silages are whole or fish parts converted into liquid form and used as additional ingredients for animal feeds. The surplus fish are also industrially composted to produce nitrogenous fertilizers [17]. Thus, to meet the demand for fish supply, different types of fisheries were developed collectively known as "capture fisheries". The fishing activities are sub-categorized as small or industrial on the basis of the species captured, gears used and the size of the industry. In view of all these, we can realize the importance of the increasing demand of fish presently [18]. The massive demand for fish led to over-exploitation not only in coastal and near shore areas, but in the deeper regions as well. All sources causing water pollution like floods, garbage, polluted water run-offs, oil spills, sunscreen wash-offs, chemical dumping and even ocean acidification affect the survival of the fish and upset the fish ecology significantly [19].

4. Roles of Pesticides in Agro-Economic System

As the human population of the world grows fast, there are competitions among the individuals for food and shelter due to limited abiotic and biotic resources. Therefore, there is a challenge to produce sufficient amount of food on limited fertile land protecting the crop from various pests. Hence, different kinds of biological and nonbiological pesticides (like organophosphates, organochlorides, carbamides etc.) are used nowadays most abundantly in pre- and post-harvesting processes to store the grains for longer periods. Studies on the impacts of different pesticides on different soil eco-systems under different temperature have shown that these can not only affect the soil ecology by providing negative impact on different species residing there but also can minimize the soil fertility [20, 21]. The most significant point with bio-pesticides is that they are easily degradable but they are very much species specific [22]. As a result these are mostly not being encouraged for use by the farmers of the agro economy based countries like India while the uses of non-biological chemicals as pesticides are very common as they are very much useful in production of grains, vegetables and fruits.

5. Effects of Pesticides on Soil and Aquatic Ecosystems

The most detrimental point with the use of chemical pesticides is that they are not degradable under normal climatic conditions [23] and also accumulate in soil. The pesticides which are not bio-degradable, are called "recalcitrant pesticides". The most common recalcitrant pesticides are DDT, parathion, aldrin, chlordane and endrin. They can survive in the soil for more than 15 years [24]. The huge groups of pesticides exert their physiological functions following twenty nine different modes of actions. They are classified as AcetylCholinesterase (AChE) Inhibitors (carbamates and organophosphates), GABA Gated chloride channel blockers (cyclodiene organochlorine, phenylpyrazoles), sodium channel modulators (pyrithrines, DDT),

nicotinic acetylcholine receptor (NACHR) competitive modulators (sulfoximines, mesoionics), NACHR allosteric modulators (spinosyns), glutamate gated chloride channel allosteric modulators (Avermectins, milbemycins), juvenile hormone mimics (like Juvenile Hormone Analogs; hydroprene, kinoprene, pyroxifen, fenoxycarb etc), non-specific multisite inhibitors (alkyl halide; methyl bromide, chloropicrin, fluoride etc) chordotonal organ trpv channel modulators (pyridine azomethine derivatives), mite growth inhibitors (etoxazole, hexythiazox), microbial disruptors of insect mid-gut membrane (Bacillus thuringiensis and the proteins produced by them), inhibitors of mitochondrial ATP synthase (organotin miticides, tetradifon), uncouplers of oxidative phosphorylation via disruption of the proton gradient (chlorfenapyr, sulfuramid), nicotinic acetylcholine receptor (NACHR) channel blockers (nereistoxin analogues), inhibitors of chitin biosynthesis, type 0 (benzoylureas), type 1 (buprofezin), dipterans moulting disruptors (cyromazine), ecdysone receptor agonists (diacylhydrazines), octopamine receptor agonists (amitraz), mitochondrial electron transport inhibitors (meti acaricides and insecticides and rotenone for complex I; beta-ketonitrile derivatives, carboxanilides for complex II; hydramethylnon, bifenazate etc. for complex III; phosphides and cyanides for complex IV), voltage-dependent sodium channel blockers (oxadiazines and semicarbazones), inhibitors of acetyl CoA carboxylase (tetronic and tetramic acid derivatives), ryanodine receptor modulators (diamides) chordotonal organ modulators - undefined target site (flonicamide) [25]. As a result they are hazardous to human population in three different ways. First, these pesticides can reduce the fertility of soil irreversibly [11]. Secondly, these get accumulated in soil and water showing bio-accumulation and bio-magnification respectively [12]. Thirdly, these pesticides can also affect both, the biotic and abiotic components of water and soil. The final destination of pesticides can be better described in flowchart format as depicted below (Fig. 1).

Thus, in all of the cases, the physiology of animals would be affected adversely due to high accumulation of pesticides both superficially and deeply i.e beneath the soil [28, 29].

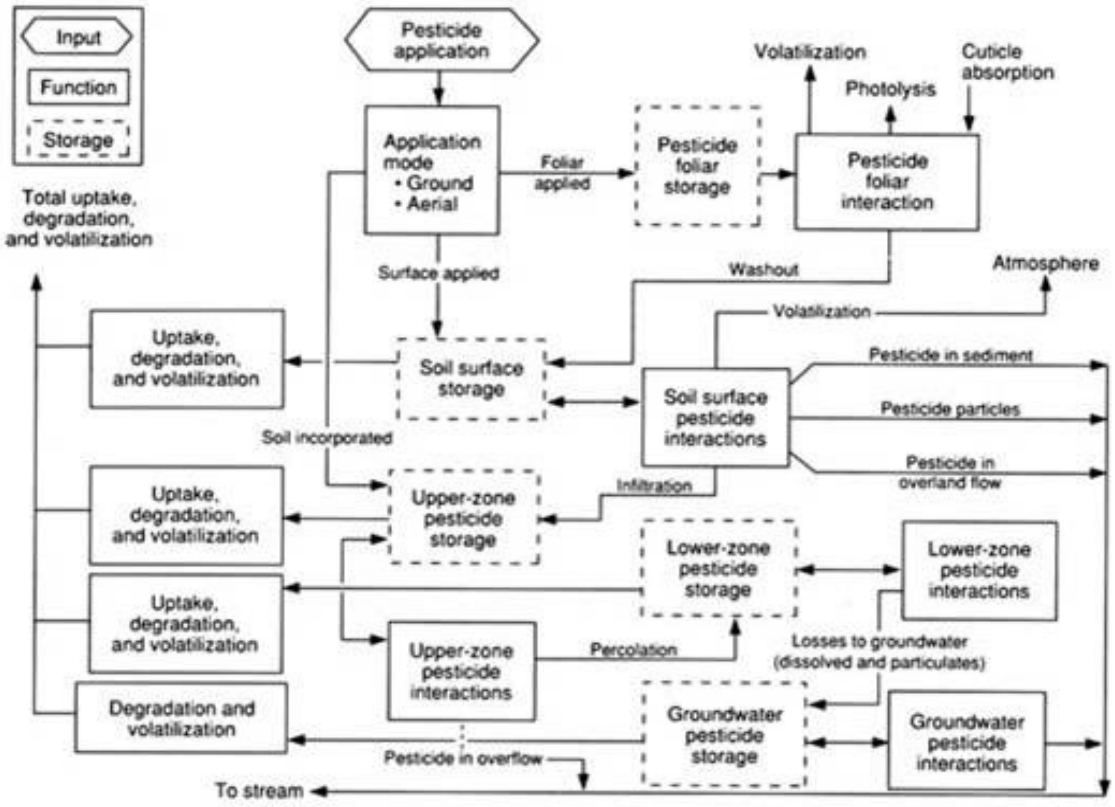

Figure 1: Pesticide transport and transformation in the soil-plant environment and the vadose zone. Source: H. H. Cheng, ed. 1990. Pesticides in the Soil Environment: Processes, Impacts, and Modeling. Soil Science Society of America Book Series No. 2. Madison, Wis.: Soil Science Society of America.

6. Blood and Body Physiology

Physiologically blood is the most significant part of the living system as it is the main circulatory component regulating and coordinating all of the metabolic processes and homeostasis in organisms. Blood transports all necessary nutrients and gases to every cell of the body and also transports back the nitrogenous wastes and carbon dioxide. The role of hemoglobin in gaseous transport is very crucial. In invertebrates where hemoglobin is present mixed in plasma as in earthworm, 30 while in vertebrates the hemoglobin is confined to red blood corpuscles (RBCs) from fishes to mammals [31]. When hemoglobin is associated with transport of different gases it can make different unstable intermediate compounds like oxy-hemoglobin (compound of oxygen and hemoglobin), carboxy-hemoglobin (carbon-mono-oxide and hemoglobin), carbamino-hemoglobin (carbon-di-oxide and hemoglobin) under normal physiological as well as clinical condition. But, the affinity of hemoglobin towards O_2 or CO_2 is dependent on a number of factors like pH, temperature etc. In the blood, when carbon dioxide reacts with water and forms carbonic acid, an increase in CO_2 results in a decrease in blood pH, resulting in hemoglobin proteins releasing their load of oxygen. Conversely, a decrease in carbon dioxide provokes an increase in pH, a process known as Bohr Effect results in hemoglobin picking up more oxygen [32]. Oxygenation of blood in the lungs displaces carbon dioxide from hemoglobin which increases the removal of carbon dioxide. This property is the Haldane effect and oxygenated blood has a reduced affinity for carbon dioxide [33].

Carbon dioxide (CO_2) generated in tissues carbonic acid and which is not highly expressed in interstitial fluid and plasma. RBC carbonic anhydrase catalyzes the conversion of dissolved CO_2 and intracellular water to carbonic acid (H_2CO_3), which spontaneously dissociates to form bicarbonate (HCO_3-) and a hydrogen ion (H+). In response to the fall of intracellular pCO_2, more CO_2 passively diffuses into the cell. The opposite process occurs in the pulmonary capillaries of the lungs when the pO_2 rises and pCO_2 falls this causes release of CO_2 from hemoglobin during oxygenation. This releases hydrogen ions from hemoglobin, increases H+ concentration within RBCs, and shifts the equilibrium towards CO_2 and water formation from bicarbonate. The subsequent decrease in intracellular bicarbonate concentration reverses chloride-bicarbonate exchange: bicarbonate moves into the cell in exchange for chloride moving out. Inward movement of bicarbonate via the Band 3 exchanger allows carbonic anhydrase to convert it to CO_2 for expiration. This process is known as Chloride Shift or Hamburger Phenomena [34]. Further, blood harbors a number of important immunogenic cells like hematocytes, granulocytes, agranulocytes in vertebrates [35, 36] and coelomocytes, adipocytes and others in invertebrates [37]. Blood and other hematological parameters is the first indicator of any kind of change in body homeostasis upon any kind of treatment and/or exposure of modulators.

Though information regarding the toxicological impact of pesticides on general physiology of target organisms is available [38-41], the same on the non-target organisms (like earthworms) are scanty [42, 43]. Further, no reports on the immunological, free radicals and energetic parameters of both fishes and earthworms upon exposure to pesticides are available presently. Therefore, we have studied the impact of sub-lethal doses of acute toxicity of organophosphates and other pesticides on different physiological and enzymatic parameters of both fishes and earthworms with a special focus on hematological parameters.

6.1. Impact of exposure of pesticides on earthworm and fishes

The general indications of effect of pesticide exposure can be manifested by the significant outrages in immunological, hematological and metabolic parameters as these help in the maintenance of body homeostasis in a proper and systematic manner. We exposed the fishes (*Heteropneustes fossilis* and *Channa punctatus*) and earthworm (*Eudrilus eugeniae*) to organophosphates and pyrethroids groups of pesticides (sublethal doses of LC_{50} value) [44] for 96 hours and the results were compared with control. As a part of our preliminary experiments, we will be expressing the results only (unpublished data).

The body homeostasis in most of the organisms changes on exposure to different pesticides [45-47], so to know the effects of organophosphates and other pesticides, levels of AST and ALT (as markers of liver function in fishes and chloragogen cell activity in earthworms), plasma Blood Urea Nitrogen (BUN), urea, creatinine and total ammonia levels (as markers

of renal function in fishes and nephridial activity in earthworms) were studied. A significant increase in all of these parameters upon pesticide exposure in a dose dependent manner was recorded.

6.2. Effect on body weight

Upon exposure, there was a significant decrease in body mass in a dose dependent manner in comparison to control in both fishes and earthworms. Cumulatively, the decreasing trends in liver function and body weight might have provoked the catabolism of the body resulting decrease in body weight.

6.3. Effect on hepatosomatic index:

We noted significantly decreasing trend of hepatosomatic index in a dose dependent manner in fishes upon exposure to pesticides when compared to control. Our results were equivocal with other reports [48]. Thus, to substantiate the results of body weight and hepatosomatic index, different circulatory metabolic parameters were also studied.

6.4. Effect on metabolic parameters

A decrease in body weight in exposed groups indicates higher rate of catabolism in animals following treatment. The circulatory level of glucose (a readymade source of energy) and protein was found significantly high in exposed groups while circulatory cholesterol was found significantly low upon treatment. The same trend of results was noted for both earthworms and fishes. Thus, it may be concluded that there is an increase in body metabolic processes upon exposure and to combat that there was a higher requirement of energy. These results were further supported by behavioral changes like increase in mobility, surfacing behavior in fishes and curling behavior in earthworms and all other behaviors related to the mobility are energy demanding. Thus, to meet the high energy requirement circulatory level of glucose was increased. Also the parameters of protein metabolism (like urea, ammonia, creatinine and BUN) were found to be significantly high. These results are equivocal with the previously available report as in carp [49]. The results of metabolic parameters would have altered the basic hematological parameters upon exposure. To find out the same, total RBC and WBC counts, Differential leukocyte counts (DLC), % hemoglobin and other related parameters were studied.

6.5. Effect on total RBC count

Significant decrease in total RBC count in fishes and that of haemocytes in earthworm was found in exposed groups.

6.6. Effect on %hemoglobin and different hemoglobin and RBC derived parameters

The % Hb content, % hematocrit value, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were found to be significantly low in a dose dependent manner upon pesticide exposure in both earthworms and fishes. Thus, lower levels of these parameters indicate that energy may get channelized to modulate different physiological processes including immunity. Similar reports of RBC count and other hematological parameters are available in other fish models like *Cyprinus carpio* and *Puntius ticto* [50].

6.7. Effect on immune parameters

In the cell-mediated immune response parameters, the TLC and DLC were found to be significantly high in exposed fishes especially the percent neutrophil level. However, in earthworms the count of coelomocyte which act as immunogenic cell was significantly high in pesticide exposed groups. This may be due to the effect of pesticides on the primary line of defense such as mucus and skin of fishes, and body wall of earthworms which got injured and to maintain the homeostasis the cell–mediated immune parameters were high. These findings are in agreement with other reports [51, 52].

6.7. Effect on free radical parameters

The free radicals are the markers of stress in any organisms which are classified as reactive oxygen species (ROS) and reactive nitrogen species (RNS). The free radicals are estimated by assaying the activities of their scavenger enzymes. The main free radical scavenging enzymes in the system are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidases (GPx). A significant increase in the level of theses enzymes in the blood of fishes and earthworms were noted upon pesticide exposure which clearly indicates that the animals had undergone physiological stress upon pesticide exposure. These results are supported by other literature [53, 54].

7. Conclusion

Depending upon our preliminary results, we may suggest that the detrimental impact of pesticides on both non-target organisms are almost similar in terms of hematological, immunological and metabolic aspects. However, further biochemical and molecular aspects are yet to be explored to identify the exact mechanism of action of organophospahates and other pesticides in earthworms and fishes.

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