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# Effect of controlled Nickel exposure on *Duttaphrynus melanostictus* oxidative stress and its management

**P. Arya Alok[1], Sushree Sangita Mohapatra[2], Taslima Begum[2]**

[1]Thinking Fox Academic Science Lab, Bhoisahi, Balasore, Odisha- 756001, India

[2]Department of Zoology, North Odisha University, Takatpur, Odisha, India

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***Abstract***

This study mainly focuses on the effect of a common heavy metal poisoning and how the oxidative stress is managed in *Duttaphrynus melanostictus* during Nickel poisoning. The subject, *Duttaphrynus melanostictus* was exposed to Nickel in regular frequency with a very small amount by creating moist environment which imitates the presence of Nickel in soil due to pollution and then the body weight analysis (by ANOVA and Post Hoc analysis (using SPSS package 16.0)) and skin coloration and pigmentation were checked at 24 hours interval to study the oxidative stress and its management by the animal's physiological system. The initial change in skin color at early stages of nickel exposure and then its restoration was observed. Similarly the loss of body weight at initial exposure and then its stabilization after some days was noted. The analysis and observations revealed that the antioxidant system of *Duttaphrynus melanostictus* is well developed to sustain against the oxidative stress due to nickel exposure.

***Keywords*:** oxidative stress, nickel poisoning, heavy metals, pigmentation



## 1. *Introduction*

Frogs, toads and other amphibians breathe through their skin and pollution can build up in their bodies. Some herpetologists think that toxic metals and pesticides were building up in the frogs' bodies, causing their second generation have defects. Antioxidant enzyme activities were largely insensitive to high urea, which accumulates during aestivation, but were inhibited by elevated KCl. Levels of reduced glutathione were also significantly lower in three organs (heart, kidney, lungs) during aestivation and all organs, except skeletal muscle, exhibited a higher oxidized/reduced glutathione ratio indicating a more oxidized state during aestivation. Energy and chemical consumption by humans are the main causes of trace element pollution in the biosphere. Non- ferrous metal industry, mining, waste disposal, pesticides, fertilizers or metal- contaminated sludge are important sources of metal dispersion in terrestrial and aquatic environment (Lepp, 1981). Soil contamination with heavy metals has become a worldwide problem, leading to losses in agricultural yield and hazardous health effects as they enter the food chain (Schickler and Caspi, 1999). Several techniques for removing heavy metal contamination from soil, water and sediment have been developed. Precipitation, ion exchange and field bioremediation using bacteria and fungi are some high impact technologies used for heavy metal removal; many factors limit the applicability of existing techniques. Another promising environmental technology still in its infancy is phyto-remediation, where living plants are applied to clean up soils or waterways. This approach exploits the ability of various plant species to thrive in high metal environments while accumulating large amounts of toxic elements; it's having advantages over existing remediation methods that include minimal site destruction and destabilisation, low environmental impact and favourable aesthetics (Nedelkoska and Doran, 2000). Nickel is widely used in industry and is common aquatic pollutant. In natural waters nickel is the dominant chemical species. It has been shown that ecological factors including various forms of anthropogenic pollution affect the physiology of animals in a given habitat. However, there has not been a lot of research investigating the changes in the morphological content of amphibians in relation to a specific pollutant in the habitat and using the skin pigmentation and coloration as a tool for bio-monitoring. In aquatic ecosystems nickel interacts with numerous inorganic and organic compounds and occurs as soluble salts adsorbed onto substances of different chemical origin. Nickel combined with other elements is present in all soils, in meteorites and is emitted from volcanoes. As for most metals, the toxicity of nickel is dependent on the route of exposure and the solubility of the nickel compound. When we come to free radicals, it is best not to think of oxygen radicals as "bad". They are generated in a

***Corresponding author***
***Full Address :***
***Department of Zoology, North Odisha University, Takatpur, Odisha, India***
***E-mail:*** *sushreee90@gmail.com*
***Contact:*** *+91-9583791971*

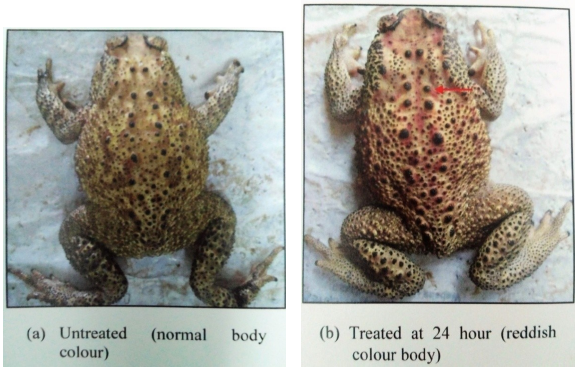
number of reactions essential to life. Despite their beneficial activities, reactive oxygen species clearly can be toxic to cells. By definition, radicals possess an unpaired electron, which makes them highly reactive and thereby able to damage macromolecules, including lipids, proteins and nucleic acids. One of the best known toxic effects of oxygen radicals is damage to cellular membranes (plasma, mitochondrial and endo-membrane systems), which is initiated by a process known as lipid peroxidation. A common target for peroxidation is unsaturated fatty acids present in membrane phospholipids. A peroxidation reaction involving a fatty acid is depicted. Pigmentation and colour change in skin of *D. melanostictus* is a significant example of this process which is to be monitored in this experiment. Members of the Food and Nutrition Board of the National Research Council in the United States recently defined a dietary antioxidant as a substance in foods which significantly decreases the adverse effects of reactive oxygen species, reactive nitrogen species, or both on normal physiological function in humans. It is recognised that this definition is somewhat narrow because maintenance of membrane stability is also a feature of antioxidant function (Dormandy. 1983) and an important antioxidant function of both vitamin A (Thumham, 1990) and zinc (Shankar and Prasad, 1998). The restoration of any initial deformities like skin pigmentation and color change and body weight alteration can be physiologically checked by functional antioxidant system of the organism itself.

## 2. Materials and methods

*Duttaphrynus melanostictus* (70 g to 120 g) were collected during night and early morning time locally in Baripada from August 2017 to November 2017. The initial mean weight of the toads was 94.6 gm. The skin color of the collected toads was normal i.e. brownish yellow (#d8b365 - in colorbrewer2 chart), as characterized in this species. They were acclimatized in normal lab condition for seven days prior to the experiment. The animals were kept in large plastic lid jars with small perforations and divided into two groups, i.e., control and experiment. The stock solution was prepared by dissolving 1gm of nickel chloride (provided from the shelf stock of North Orissa University, Takatpur, Baripada) in 1000ml of distilled water. The solution was used to wet the absorbent cottons to be used as the bed for the toads imitating the damp soil environment with nickel contamination. The lids of the jars were kept open for 30 minutes time to time at 12 hours interval just to enter the fresh oxygen and whenever needed, the solution is reintroduced to keep the cotton moistened. 15 numbers of toads are reared in this condition except 3 control toads which were kept without the nickel solution. The animals were then monitored at 24 hours, 48 hours and 72 hours; body weight (milligram scale) and change in skin coloration and pigmentation (using diverging and then sequential colour scales, from Colorbrewer2.org) was noticed.

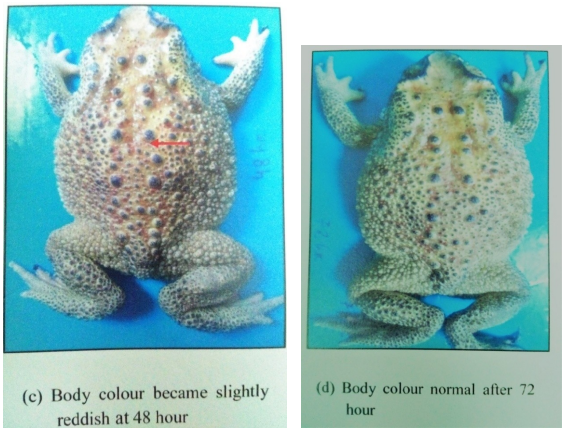
## 3. Results

Toxicity of nickel on morphology of *Duttaphrynus melanostictus:* The toxic effect of nickel against *D. melanostictus* was recorded at 0 hour, 24 hour 48 hour and 72 hour *(Fig- a, b, c, d).* The study revealed that lower concentration was enough to cause visible changes in morphology. Small red spots were developed over the body in 24 hour exposure to nickel. First the body colour became yellow (#ffeda0) and then more reddish (#fdae6b) in 24 hour of exposure to nickel. It gradually changed to normal skin color in 48 hour and 72 hour and the tympanum became white in 72 hour. The toads which were retained untreated with nickel and taken as control were found with no change in color or any variation of pigments. The behavioural responses of the toad to toxicants varied with metals which was another aspect of the study.



(a) Untreated (normal body colour)

(b) Treated at 24 hour (reddish colour body)



(c) Body colour became slightly reddish at 48 hour

(d) Body colour normal after 72 hour

Comparison of body weight in *Duttaphrynus melanostictus* treated with nickel at different time intervals: Body weight of *Duttaphynus melanostictus* exposed to nickel were 94.6 ± 17.11641 before exposure (BE) and then reduced to 92.46 ± 18.7344 after exposure (AE) in 24 hour, 89.93 ± 18.8014 after exposure in 48 hour and 87.8 ± 18.6593 after exposure in 72 hour. In other words body weight of *Duttaphrynus melanostictus* after exposure decreased maximum in 72 hour. Whereas the organisms retained as controls were found with no such significant change in body weight (averaging 0.34 gm reduction after 72 hours), which seemed to be normal in lab captive condition, hence not taken for further statistical analysis or observation. Only nickel exposed test subjects were taken for statistical data (Table-1).

**Table- 1: Comparison of body weight in *Duttaphrynus melanostictus* treated with nickel at different time intervals. Value is expressed in mean± SD**

| Serial no. | 0 hours | 24 hours | 48 hours | 72 hours |
|---|---|---|---|---|
| 1 | 100 | 98 | 95 | 93 |
| 2 | 115 | 113 | 111 | 109 |
| 3 | 75 | 73 | 73 | 70 |
| 4 | 98 | 96 | 94 | 91 |
| 5 | 95 | 93 | 90 | 88 |
| 6 | 118 | 114 | 112 | 110 |
| 7 | 102 | 99 | 97 | 96 |
| 8 | 98 | 95 | 93 | 90 |
| 9 | 77 | 74 | 70 | 69 |
| 10 | 82 | 79 | 75 | 73 |
| 11 | 70 | 65 | 61 | 60 |
| 12 | 120 | 115 | 113 | 110 |
| 13 | 75 | 70 | 68 | 66 |
| 14 | 130 | 125 | 121 | 119 |
| 15 | 83 | 78 | 76 | 73 |
| Mean ± SD | 94.6 ± 17.11641 | 92.46 ± 18.7344 | 89.93 ± 18.8014 | 87.8 ± 18.6593 |

## *Discussion:*

As experimented with 15 toads in a specified environment of nickel poisoning the results revealed some significant results to be documented. The initial mean weight of toads was 94.6 g. After days of exposure to nickel treated environment it was found that the body weight of *Duttaphrynus melanostictus* significantly decreased than that of controls. At 24 hour, 48 hour and 72 hour interval of nickel exposure time the mean weight fall were recorded as 92.46 ± 18.7344, 89.93 ± 18.8014 and 87.8 ± 18.6593 respectively. Further the continued exposure for the period of 30 days was done to observe prolonged effect of nickel on the body weight of *Duttaphrynus melanostictus*. In 9 toads the body weight got stabilized after 13-14 days of exposure and they got a constant weight; and in other larger toads it took up to 24 days to get weight stabilization. This gave a clear indication that the Nickel contamination surely affected on the physiology that was reflected on the body weight; but after some days they got stabilized with no more weight loss. The change in animals' body colour was another visible parameter which was measured by comparing the skin color with the color pellet chart (from Colorbrewer2.org) by using diverging and then sequential colour scales. Initially, the skin color changed to reddish at 24 hours, then slightly reddish at 48 hours and finally back to normal color of the skin. This signified the active antioxidant activity of the toad that could bring back the skin color to normal by dealing with the nickel poisoning. The symptoms could not persist for more than 40 days with this concentration of exposure to contaminant; perhaps the toads acclimatized themselves to the controlled test environment.

## *Conclusion:*

Amphibian populations are facing with array environmental problems, including inter-specific competition, climate change and over-harvesting for the pet and food trades. Unless we act quickly, amphibian species will continue to disappear, resulting in irreversible consequences to the planet's ecosystems and to humans. Metals are biologically important. If the threshold concentration of the metal increases in the environment, they may interfere with the metabolic activity of organisms. In the presenting study, an attempt was made to investigate the effect of nickel on body weight and change in skin pigmentation and colouration. It was examined that this concentration (1gm/Litre) of nickel exposure, that to be by creating moist environment to breathe through the skin and nostrils do minimal yet significant damage to the toad which got managed by the toad's antioxidant system, but above this concentration what will happen that is the question to be asked. Another aspect is that, if the nickel poisoning happens through oral intake of contaminated food sources of *Duttaphynus melanostictus* than how it will deal with the toxic systems by the help of its own body antioxidant system; this can only be supervised by oral dose supervision and experimentation on internal organs and SOD, CAT-activity in the physiology of the toad.

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