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Effect of Nickel on the Microflora of Gill, Gut and Skin of *Clarias gariepinus*

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Abstract: A study was carried on the effect of nickel exposure on the miroflora of the skin and gut of African catfish, *Clarias gariepinus*. *C. gariepinus* of different stages was applied with nickel and monitored every 24 h over a period of 672 h in wooden vats under a laboratory condition. Eleven adult samples, thirty six post juvenile and thirty one juvenile samples were analyzed for the isolation of microflora using biochemical test. Both gram +ve and gram -ve bacteria were isolated. The gram -ve bacteria isolated were mainly rods and members of the Family Enterobacteriaceae including *Proteus* and *Serratia*. These species were more widely distributed in and more frequently isolated from the gut regions than the gram +ve bacteria which were represented by *Streptococcus* and *Staphylococcus* species. *Proteus* species and *Streptococcus* species isolated from the gut extracts were also found in the skin region. *Bacillus* species and *Pseudomonas* species were also found in both the skin and gut regions of the fish. The number and types of microbes in the skin and gut regions are similar.

Key words: Proteus, Serratia, species, nickel, *Staphylococcus* species, Nigeria

INTRODUCTION

The indigenous microflora of fish has been described for many purposes. This includes descriptions of microbial spoilage, relationship between environment and fish microflora (Horsley, 1977) the monitoring of changes in fish farms the nutritional role of the intestinal microflora (Moriarty, 1990) and the antibiotic resistance profile of the indigenous flora and the disease-preventive role of bacterial strains known as probiotics.

Contamination of aquatic system with heavy metals has become a matter of great concern over the past few decades. The source of heavy metals in aquatic environment may be through natural or anthropogenic sources. Natural sources include weathering of minerals and soils (Merian, 1991) while anthropogenic inputs are mainly from industrial effluents, domestic effluents, rural and urban storm, water run-off and spoil heaps (Agbozu and Ekweozor, 2001). The effect of heavy metals has consequently received the attention of several researchers (Annune and Iyaniwura, 1993; Baranowska-Bosiacka *et al*., 2000; Olojo *et al*., 2005; Van Vuren *et al*., 1994). However, information on the effect of metals on the microflora of fish is scarce.

Nickel is a naturally occurring metal existing in different basic ores namely arsenide, laterite, silicate and sulphate (Galvin, 1996). It is a natural ubiquitous element of the earth and in water (Snodgrass, 1980). Inhaled insoluble nickel increases the risk of cancer or gastrointestinal symptoms. Nickel inhalation exposure increases susceptibility to respiratory infection, allergic contact, dermatitis and pulmonary edema (Snodgrass, 1980).

Fish are able to accumulate and retain heavy metals from their environment (Pagenkopf, 1983). The accumulation of metals in tissues of fish is dependent upon exposure concentrations and duration as well as other factors such as salinity, temperature, hardness and metabolism of the animals (Allen, 1995). Fishes are bathed in aqueous solution of microbes. These microbes have the choice of living in association with the potential host (intestinal tract, gills or skins) or not.

Organisms may colonize the surface of the fish thereby, becoming part of the resident microflora. The presence of these microflora inhibits the arrival and subsequent colonization by other organisms that may be pathogenic to the fish. The microflora of living fish depends on the microbial contents of the water in which the fish live.

Fish microflora includes bacteria such as species of *Pseudomonas*, *Alcaligenes*, *Vibrio*, *Micrococcus*, *Baccillus*, etc. Surrounding bacteria are continuously ingested at a high rate from the culture water either with the feed or when the fish is drinking causing a natural

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interaction between the microbiota of the ambient environment and the live food. Both human and animals need a normal bacterial flora in the digestive tract for protection against disease causing bacteria and production of compounds that are important for a well functioning digestion.

Probiotics are non-pathogenic and non-toxigenic organisms which retain viability during storage and survive passage through the small intestine and stomach. They benefit the host when grown in a particular microenvironment by inhibiting or preventing the growth of other biological organisms within the same microenvironment.

The prebiotics bacteria are the non-digestible food ingredients that selectively stimulate the growth and activity of beneficial microorganisms already present in the colons of humans (Gibson and Roberfroid, 1995). The ingestion of beneficial bacteria leads to colonization of the gut with the added strain and this strengthens the gastrointestinal barrier to disease.

In terms of microbial population, the stomach has very low bacterial numbers due to low environmental pH (Cummings *et al*., 1989). The bacterial population within the gastrointestinal tract of mammals constitutes a metabolically active organ that act as a barrier to infection by exogenous pathogenic microorganisms. While gastrointestinal microflora presents a microbial-based barrier to invading organisms, pathogens become established when the integrity of the microbes is under stress, antibiotic treatment or physiological alterations.

African catfish, *Clarias gariepinus* is an omnivore fresh water fish. It is suitable for culture because of its tolerance to adverse environmental conditions. It is of great commercial importance because it is the most common fresh water fish widely consumed in Nigeria (Olaifa *et al*., 2004). The numbers and types of bacteria inhabiting different portions of the digestive tract vary as a result of the pH and oxygen level within each region.

Generally, there is a progressive increase in the number of bacteria found in the gut with the stomach having the lowest numbers as a result of the low pH, the small intestine has a large bacterial load while the most heavily colonized region is the colon (Gibson and Roberfroid, 1995).

MATERIALS AND METHODS

Thirty one juveniles of African catfish, *Clarias gariepinus* (mean Total Body Length TB Lin cm = 10.0±2.0; mean body weight, BW in grams 0.03±0.02 and 36 post-juveniles (mean TBL cm 19±2.3; mean BW in grams 26±2.4 and eleven adult (mean TBL in cm 38±7.0; mean BW in grams 580±5.0 were obtained from a local fish farm. Fish were fed with commercially formulated floating feed and kept in three vats for 2 weeks to acclimatize before the commencement of the experiment.

The fish were divided into nine experimental vats (5×10×4 ft) containing fresh water (pH 7.7) for 72 h before introducing the heavy metal. Dissolved nickel was introduced into the water in the form of nickel bicarbonate. Two concentrations of nickel $T_1 = 0.06$ mg L^{-1} and $T_2 = 0.08$ mg L^{-1} were used for each stage of the fish viz: juvenile, post juvenile and adult each having two replicates. The experiment lasted for 4 weeks.

Fish specimens were randomly selected, two from each vat at 2 weeks interval and transported to the laboratory for isolation, identification and biochemical characterization of microflora of the gut and skin.

Isolation from the skin was made while the fishes were alive. The fishes used were taken with hand-net and seized with the hand with sterile gloves. A sterile swab was rubbed along the skin. The swab immediately cultured on Nutrient Agar which had been prepared afore time. The swab spot on the medium was streaked out using a sterile inoculating loop. The plate was kept for incubation at room temperature in an inverted position for 24 h.

The fish sample was dissected to open up the stomach. With the aid of a sterile blade, the stomach was cut into two and two pairs of sterile forceps were used to turn the stomach inside-out. A sterile swab was used to rub the exposed inner surface of the stomach and cultured on nutrient agar after which streaking was carried out using a sterile inoculating loop. The plates were then incubated at room temperature for 24 h. Characterization of the organisms was based on two criteria:

- Cultural and morphological characteristics of the colonies
- Biochemical characteristics

All the isolates were cultured on the prepared medium in duplicates and incubated aerobically at 37°C. The colonies were observed on the agar medium plates while the cell morphology was observed microscopically after staining. Various biochemical tests were carried out on the bacterial isolates for possible identification. One milliliter of both culture of each isolate was used for all the tests except otherwise stated.

Gram staining was carried out by the methods of Harrigan and McCance (1966). Motility, catalase and oxidase tests were carried out on the isolates using the methods stated by Seeley and VanDemark (1972). A delayed reaction was recorded as negative.

RESULTS AND DISCUSSION

Skin: Table 1 and 2 show the result of various biochemical test carried out for the identification of the bacterial isolates. In skin of *C. gariepinus*, only *Bacillus macerans* was isolated in T2 on day 14 but did not occur on day 28 (Table 3). Bacteria such as *Streptococcus faecium*, *S. zymogenes*, *Bacillus cereus* and *Proteus mirabilis* only occurred in the natural microflora but did not occur in *C. gariepinus* exposed to the two concentrations of nickel bicarbonate. Some bacteria such as *B. cereus*, *P. purela*, *Micrococcus acidiophilus*, *Aerobacter aerogenes*, *Proteus morganii*, *P. mirabilis* (day 14) and *Pseudomonas fragii*, *P. putida*, *P. florescence* and *A. aerogenes* (day 28) occurred in 0.06 mg L^{-1} Nibut were not found in 0.08 mg L^{-1} Ni.

On day 14, four bacteria were isolated from the skin microflora of *C. gariepinus* in T2: *B. macerans*, *Streptococcus zymogenes*, *Pseudomonas florescence* and *P. fragii* while on day 28, *Pseudomonas gellucidium* and *Proteus vulgaricus* were isolated.

Gut: In the natural gut microflora, only *Pseudomonas faecium* was isolated in T2 day 14. Other bacteria constituting the natural microflora did not occur in the two treatments of Ni on day 14 and 28. *Enterobacter aerogenes* occurred in the two treatments of Ni while the following occurred in T1 only: *S. zymogenes* (day 14) and *Pseudomonas purela*, *P. mullei*, *Serratia marscences* and

Table 1: Biochemical characteristics and identification of bacterial isolates from the skin and gut of *Clarias gariepinus* (Result 1)

Laboratory cod	Gram stain	Shape	Motility	Catalase	Oxidase	Coagulase	Urease	Indole	Methyl Red	Voges-ProsKaner	Gelatin hyrolysis	Starch hydrolysis	Pigmentation	Oxygen relationship	H_2S production	Casesin hydrolysis
SC	+	R	+	+	+	-	-	-	-	-	+	+	-	a	-	+
ST_2	+	R	+	+	+	-	-	-	-	-	+	+	-	a	-	+
GC	-	R	+	+	+	-	-	-	+	-	-	+	-	a	-	-
ST_1	+	R	+	+	+	-	+	-	-	-	+	+	+	a	-	+
GC	-	R	+	+	+	-	+	+	+	-	+	+	-	a	+	+
ST_2	+	S	-	-	-	-	+	+	-	-	+	+	-	a	+	+
ST_1	-	R	+	+	+	-	-	-	+	-	-	-	+	a	+	-
ST_2	-	R	+	+	+	-	+	+	+	-	+	-	+	a	-	+
ST_1	-	R	+	+	+	-	-	+	-	-	-	+	-	a	-	-
ST_2	-	R	+	+	+	-	+	+	+	-	+	+	-	a	+	+
GT_2	-	R	-	+	+	-	-	+	-	+	-	-	+	a	+	-
GT_2	-	R	+	+	-	-	+	-	-	+	+	+	-	a	-	+
SC	+	S	-	+	-	-	+	+	-	-	+	+	+	a	+	-
ST_1	+	C	+	+	+	-	-	-	-	-	+	-	-	a	-	+
GT_1	-	R	+	+	-	-	+	-	-	+	+	+	-	a	-	+
GT_1	+	S	-	-	-	-	+	+	-	-	+	+	-	a	+	+
GT_2	-	R	+	+	+	-	+	+	+	-	+	-	+	a	-	+
ST_1	-	R	+	+	-	-	+	-	-	+	+	+	-	a	-	+
SC	+	R	+	+	+	-	-	-	-	-	+	+	-	a	-	+
GT_2	+	R	+	+	-	-	-	-	-	-	-	+	-	a	-	-
ST_1	-	R	+	+	-	-	-	+	+	-	+	+	-	a	+	+
GC	-	R	+	+	+	-	+	+	+	-	+	-	+	a	-	+

Sugar fermentation

Fructose	Sucrose	Lactose	Mannitol	Arabiniose	Xylose	Dulcitol	Raffinose	Glucose	Maltose	Adonitol	Probable identification
A/G	A/G	A/G	d	A/G	A/G	A/G	A/G	A/G	A/G	A/G	*Bacillus macerans*
A/G	A/G	A/G	d	A/G	A/G	A/G	A/G	A/G	A/G	A/G	*Bacillus macerans*
A/G	A/G	A/G	A/G	A/G	d	A/G	A/G	A/G	A/G	A/G	*Proteus morganii*
A/G	A/G	A/G	A/G	A/G	A/G	d	A/G	A/G	A/G	A/G	*Bacillus cereus*
A	A	A	A	A	d	A	d	A	A	A	*Pseudomonas fragii*
A	A	A	A	A	d	A	A	A	A	A	*Streptococcus zymogenes*
A	A	A	-	-	A	A	A	d	A	A	*Pseudomonas purela*
A	A	A	A	-	d	A	A	-	A	A	*Pseudomonas florescences*
A/G	A/G	A/G	A/G	A/G	d	A/G	A/G	A/G	A/G	A/G	*Proteus morganii*
A	A	A	A	A	d	A	d	A	A	A	*Pseudomonas fragii*
A	A	A	A	-	-	A	A	d	A	A	*Xanthomonas maltophyla*
A/G	A/G	A/G	-	d	A/G	A/G	A/G	-	A/G	A/G	*Enterobacter aerogenes*
A	A	A	A	A	A	A	A	A	A	A	*Streptococcus faecium*
A	A	A	d	A	A	d	A	A	A	A	*Micrococcus acidiophilus*
A/G	A/G	A/G	-	d	A/G	A/G	A/G	-	A/G	A/G	*Enterobacter aerogenes*
A	A	A	A	A	A	A	A	A	A	A	*Streptococcus zymogenes*
A	A	A	A	-	d	A	A	-	A	A	*Pseudomonas florescences*
A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	d	A/G	A/G	*Aerobacter aerogenes*
A/G	A/G	A/G	d	A/G	A/G	A/G	A/G	A/G	A/G	A/G	*Bacillus macerans*
A	A	A	A	A	A	A	A	A	A	A	*Bacillus subtilis*
A/G	A/G	A/G	A/G	A	A	A/G	A/G	A/G	A	A	*Proteus mirabilis*
A	A	A	A	-	d	A	A	-	A	A	*Pseudomonas florescences*

Table 2: Biochemical characteristics and identification of bacterial isolates from the skin and gut of *Clarias gariepinus* (Result 2)

Laboratory cod	Gram stain	Shape	Motility	Catalase	Oxidase	Coagulase	Urease	Indole	Methyl Red	Voges-ProsKaner	Gelatin hyrolysis	Starch hydrolysis	Pigmentation	Oxygen relationship	H_2S production	Casesin hydrolysis
ST_1	-	R	+	+	+	-	+	+	+	-	+	+	-	a	-	+
ST_1	-	R	+	+	-	-	+	-	-	-	+	+	-	a	-	+
ST_1	-	R	+	-	-	-	-	+	+	-	-	+	+	a	-	-
GT_1	-	R	+	+	+	-	-	-	+	-	-	-	+	a	+	-
ST_1	-	R	+	+	+	-	+	+	+	-	+	-	+	a	-	+
GT_1	-	R	+	+	-	-	-	-	+	+	+	+	-	a	-	-
GT_1	-	R	+	+	-	-	+	-	-	+	-	-	+	a	-	-
GT_1	-	R	+	+	-	-	+	-	-	-	+	+	-	a	-	+
ST_2	-	R	-	-	+	-	+	-	+	+	+	+	-	a	-	+
GT_2	+	C	-	+	-	-	+	-	-	-	-	+	-	a	-	+
GT_2	+	S	-	+	-	-	+	+	-	-	+	+	+	a	+	-
ST_2	-	R	+	+	+	-	+	+	+	-	-	+	-	a	-	-
GC	-	R	+	+	+	-	+	-	-	-	+	+	+	a	+	+
SC	+	R	+	+	+	-	+	-	-	-	+	+	+	a	-	+
GC	-	R	+	+	+	-	-	+	-	-	+	+	+	a	-	-
SC	-	R	+	+	-	-	-	+	+	-	-	+	-	a	-	+
GC	+	R	+	+	+	-	-	-	-	-	+	+	-	a	-	+
SC	+	S	-	-	-	-	+	+	-	-	+	+	-	a	+	+

Sugar fermentation												
Fructose	Sucrose	Lactose	Mannitol	Arabiniose	Xylose	Dulcitol	Raffinose	Glucose	Maltose	Adonitol	Probable identification
A	A	A	A	A	d	A	d	A	A	A	*Pseudomonas fragii*
A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	d	A	A/G	*Aerobacter aerogenes*
A	A	A	-	-	d	A	A	A	A	A	*Pseudomonas putida*
A	A	A	-	-	A	A	A	d	A	A	*Pseudomonas purela*
A	A	A	A	-	d	A	A	-	A	A	*Pseudomonas florescenc*
A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	A/G	*Serratia marscences*
A	A	A	A	-	d	A	A	-	d	A	*Pseudomonas mullei*
A/G	A/G	A/G	-	d	A/G	A/G	A/G	-	A/G	A/G	*Enterobacter aerogenes*
A	A	A	A	A	d	d	A	A	A	A	*Pseudomonas gellucidiun*
A	A	A	A	A	d	A	A	A	A	A	*Staphylococcus parasiticus*
A	A	A	A	A	A	A	A	A	A	A	*Streptococcus faecium*
A/G	A/G	A/G	-	d	A/G	A/G	A/G	-	A/G	A/G	*Proteus vulgaricus*
A	A	A	A	A	A	A	A	A	A	A	*Pseudomonas aureginosa*
A/G	A/G	A/G	A/G	A/G	A/G	d	A/G	A/G	A/G	A/G	*Bacillus cereus*
A/G	A/G	A/G	A/G	A/G	d	A/G	A/G	A/G	A/G	A/G	*Proteus morganii*
A/G	A/G	A/G	A/G	A	A	A/G	A/G	A/G	A	A	*Proteus mirabilis*
A/G	A/G	A/G	d	A/G	A/G	A/G	A/G	A/G	A/G	A/G	*Bacillus macerans*
A	A	A	A	A	A	A	A	A	A	A	*Streptococcus zymogenes*

Table 3: Isolates and sites of isolation in *Clarias gariepinus* treated with Ti (0.6 mg L^{-1} $NiHBCO_3$) and T2 (0.8 mg L^{-1} $NiHBCO_3$)

	Control		T1		T2	
Isolates	Skin	Gut	Skin	Gut	Skin	Gut
Aerobacter aerogenes	+	+	+		+	
Bacillus cereus	+		+			
B. macerans	+	+			+	
B. subtilis						+
Enterobacter aerogenes	+			+	+	+
Micrococcus acidiophilus	+		+			
Proteus mirabilis	+		+			
Proteus morganii	+		+			
Proteus vulgaricus					+	
Pseudomonas aeruginosa		+				
P. florescences		+	+		+	+
P. fragii		+	+	+	+	
P. gellucidium					+	
P. mullei				+		
P. purela			+	+		
P. putida			+			
Serratia marscences				+		
Staphilococcus parasiticus						+
Streptococcus faecium	+					+
S. zymogenes	+			+	+	
Xanthomonas maltophila	+					+

Enterobacter aerogenes (day 28). *Xanthomonas maltophila* was the only entrant in T2 (day 14) while *Staphilococcus parasiticus* and *Streptococcus faecium* occurred in T2 (day 28). Exposure of *C. gariepinus* to Ni toxicity had a profound effect on the composition and abundance of skin and gut microflora (Table 4-6).

Tam and Wong (1995) stated that heavy metal contamination in aquatic environment is a critical concern due to the toxicity of metals and their accumulation in aquatic habitats. Although, metals such as Copper (Cu) and Zinc (Zn) are generally regarded as essential trace metals in view of their metabolic activities in organisms, other metals like Cadmium (Cd), Lead (Pb), Nickel (Ni) and Mercury (Hg) exhibit extreme toxicity even at trace levels (Merian, 1991; DWAF, 1996).

Metal contamination in aquatic ecosystems has long been recognized as a serious pollution problem. Most of the essential metals are toxic when supplied in high concentration at the optimum levels. Among the aquatic fauna, fish is the most susceptible to heavy metal toxicants (Nwaedozie, 1998) and so are more vulnerable to metal contamination than any other aquatic fauna.

Bacteria causing spoilage of fish are part of the natural flora and predominant among them are *Pseudomonas* sp., *Achromobacter* sp., *Flavobacterium* sp., *Micrococcus* sp. and *Bacillus* sp. Some of these bacteria can grow at low temperature, first on the surface and later penetrate into the flesh or tissue. Different kinds of bacteria are found in the intestinal system which are known as gut flora: when the balance of the gut flora is disturbed, it results in unpleasant gastrointestinal symptoms in man and aquatic organisms.

Table 4: Abundance of bacteria present on skin of *Clarias gariepinus* exposed to two concentrations of Ni on Day 14

		Bacteria isolated						
Days	Ni concentration	*Aerobacter aerogenes*	*Bacillus cereus*	*B. macerans*	*Micrococcus acidiophilus*	*Proteus mirabilis*	*Proteus morganii*	*Proteus vulgaricus*
14	Control	-	-	5	-	-	-	-
	T1: 0.06 mg L^{-1}	2	8	-	1	2	2	-
	T2: 0.08 mg L^{-1}	-	-	5	-	-	-	-
28	Control	-	8	-	-	5	-	-
	T 1 : 0.06 mg L^{-1}	2	-	-	-	-	-	-
	T2: 0.08 mg L^{-1}	-	-	-	-	-	-	5

		Bacteria isolated						
Days	Ni concentration	*P. florescences*	*P. fragii*	*P. gellucidium*	*P. purela*	*P. putida*	*Streptococcus faecium*	*S. zymogenes*
14	Control	-	-	-	-	-	12	-
	T1: 0.06 mg L^{-1}	-	-	-	5	-	-	-
	T2: 0.08 mg L^{-1}	15	3	-	-	-	-	12
28	Control	-	-	-	-	-	-	2
	T1: 0.06 mg L^{-1}	15	3	-	-	10	-	-
	T2: 0.08 mg L^{-1}	-	-	5	-	-	-	-

Table 5: Abundance of bacteria present on gut of *Clarias gariepinus* exposed to two concentrations of Ni

Days	Ni concn	*B. macerans*	*Enterobacter aerogenes*	*Proteus morganii*	*Pseudomonas aeruginosa*	*P. faecium*	*P. fragii*
14	Control	-	-	2	-	15	3
	T1: 0.06 mg L^{-1}	-	2	-	-	-	-
	T2: 0.08 mg L^{-1}	-	2	-	-	15	-
28	Control	5	-	-	6	-	-
	T1: 0.06 mg L^{-1}	-	2	-	-	-	-
	T2: 0.08 mg L^{-1}	-	-	-	-	-	-

Days	Ni concn	*P. mullei*	*P. purela*	*Serratia marscences*	*Staphylococcus parasiticus*	*Streptococcus faecium*	*S. zymogenes*	*Xanthomonas maltophila*
14	Control	-	-	-	-	-	-	-
	T1: 0.06 mg L^{-1}	-	-	-	-	-	2	-
	T2: 0.08 mg L^{-1}	-	-	-	-	-	-	1
28	Control	-	-	-	-	-	-	-
	T1: 0.06 mg L^{-1}	3	5	2	-	-	-	-
	T2: 0.08 mg L^{-1}	-	-	-	20	12	-	-

Table 6: Becterial counts and physiologic identification of strains isolated from TSA plates sampled from *clarias gariepinus* exposed to 0.08 mg L^{-1} of nikel bicarbonate (T_1)

Days	Ni concn	*B. macerans*	*Enterobacter aerogenes*	*Proteus morganii*	*Pseudomonas aeruginosa*	*P. faecium*	*P. fragii*
14	Control	-	-	2	-	15	3
	T1: 0.06 mg L^{-1}	-	2	-	-	-	-
	T2: 0.08 mg L^{-1}	-	2	-	-	15	-
28	Control	5	-	-	6	-	-
	T1: 0.06 mg L^{-1}	-	2	-	-	-	-
	T2: 0.08 mg L^{-1}	-	-	-	-	-	-

Days	Ni concn	*P. mullei*	*P. purela*	*Serratia marscences*	*Staphylococcus parasiticus*	*Streptococcus faecium*	*S. zymogenes*	*Xanthomonas maltophila*
14	Control	-	-	-	-	-	-	-
	T1: 0.06 mg L^{-1}	-	-	-	-	-	2	-
	T2: 0.08 mg L^{-1}	-	-	-	-	-	-	1
28	Control	-	-	-	-	-	-	-
	T1: 0.06 mg L^{-1}	3	5	2	-	-	-	-
	T2: 0.08 mg L^{-1}	-	-	-	20	12	-	-

Nickel is a natural ubiquitous element of the earth and in water (Snodgrass, 1980). Toxicity is the capacity to cause injury to a living organism defined with reference to the quality of chemical administered. Nickel may be absorbed as the soluble Nickel ion (Ni^{+2}) while sparingly soluble nickel compound may be phagocytized. Nickel ions tend to be soluble at pH of 6.5 and above 6.7 they mostly form insoluble nickel hydroxides (Dallas and Day, 1993).

In aquatic ecosystems, dissolved Ni concentrations are generally between 0.005 and 0.010 mg L^{-1} (Galvin, 1996). The toxicity of Ni to aquatic life has been shown to vary significantly with species of organisms, pH and water hardness (Dallas and Day, 1993; Birge and Black, 1980). Nickel toxicity is generally low (Khangarot and Ray, 1990) but elevated concentrations have been reported to cause sub lethal effects. Nickel is readily distributed throughout the body but may be affected by route of

exposure, the chemical form and the time after exposure (Coogan *et al*., 1989). The chemical form and its site of deposition in the lungs will affect the extent of absorption (ATSDR, 1988). The kidney and lungs are the primary sites of accumulation of nickel, other organs such as the spleen, liver, heart and testes may also accumulate the metal to a much lesser rate. Much of the toxicity of nickel may be associated with its interference in the physiological processes of manganese, zinc, calcium and magnesium (Coogan *et al*., 1989).

Nickel may be removed from portions of the respiratory tract through mucociliary transport resulting in the material entering the gastrointestinal tract. Although, nickel is poorly absorbed from the gastrointestinal tract, dietary exposure and exposure via drinking water provide most of the intake of nickel and nickel compounds (Coogan *et al*., 1989).

Majority of *Bacillus* species have generally been thought to be unsuitable for colonization of the gut due to their instability in the harsh pH environment of the bile, particularly human bile. *Bacillus coagulans* was found to survive and colonize the gastrointestinal tract such as bile environment and grown in a low pH range. The human bile environment is different from the bile environment of animal models and there has not been any accurate description of *B. coagulans* growth in human gastrointestinal tract.

The purified population of *B. coagulans* has an optimal growth temperature of <45°C for example; the isolated population of *B. coagulans* has an optimal growth temperature of 20°C, more preferably 36°C and most preferably 37°C. The strain grows at a low pH such as pH conditions found in the gastrointestinal tract of a mammal. Another experiment was carried out on the microbiological state of fish feed, water and skin of European catfish, *Silurus glanis* fry during intensive rearing in a pond. The number of heterotrophic bacteria cultured on broth agar at a temperature of 22 and 37°C, heterotrophic bacteria cultured on TGY medium at a temperature of 25°C. Bacteria such as *Pseudomonas flourescens*, *Aeromonas hydrophila* and *Pseudomonas aeruginosa* were found in the water. *Pseudomonas fluorescens* was found in the feed while *Pseudomonas aeruginosa* and *Aeromonas hydrophila* were not observed in the feed. Potentially pathogenic bacteria were observed in numbers ranging from several to several dozen on the skin surface (Iwona and Monika, 2004).

Itoi *et al*. (2006), carried out a study on chitinolytic bacteria in the intestinal tract of Japanese coastal fishes. These bacteria from several coastal fish species were screened on 1/2o PYBG medium containing 0.2% colloidal chitin and 361 bacteria capable of decomposing colloidal chitin were isolated. These isolates were screened on media containing either 0.5% alpha-chitin resulting in the identification of 31 β-chitinolytic and 275 β-chitinolytic bacterial isolates. Phylogenetic that is the β-chitinolytic bacteria belonging to the Vibrionaceae formed a separate cluster from the non-β-chitinolytic bacteria in the Vibrionaceae.

CONCLUSION

It is conclude that the purpose of this study was to investigate the effect of nickel on the gill, gut and skin of *Clarias gariepinus*. This is important to be able to explore the use of these microflora as potential indicators of metals pollution in aquatic environment. Also, the reaction of the microflora of *Clarias gariepinus* under stressor agents such as exposure to nickel.

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