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Articles and Statements

Bioaccumulation pattern of trace metals in commercially important crustaceans in Indian Sundarbans

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Abstract

We measured the concentrations of Pb and Cd, in muscle tissue of 3 commercially important crustacean species (*Penaeus monodon, Fenneropenaeus indicus* and *Metapenaeus brevicornis*) collected from the UNESCO declared world heritage site Indian Sundarbans. The Indian Sundarbans estuarine system is recognized as one of the most diversified and productive ecosystems in the world located at the confluence of Hooghly-Matla estuarine complex. Due to negative pressures like intense industrialization, urbanization and increase of anthropogenic activities in recent era, this ecosystem is getting contaminated with toxic heavy metals which vary with seasons. Significant variation of heavy metals in muscle tissue of 3 commercially important crustaceans collected from four different sampling stations (2 in central and 2 in western sector) of Indian Sundarbans were observed. Inductively coupled plasma – mass spectrometer was used to study the level of metals in tissues of selected species. The concentration of trace metals accumulated in all the muscle tissues of selected species followed the order Pb> Cd. The distribution of metals exhibited significant spatial variation and followed the order station 1 >station 2 > station 3 > station 4, which may be due to variable degree of contamination in different location ($p < 0.01$) indicating the adverse impact of industrialization and urbanization on the edible crustaceans community. When compared with the recommended value of World health Organization (WHO, 1989) in context to consumption of seafood, metal concentration in all the crustaceans showed higher value, the only exception was Sajnekhali (Station 4) for *Metapenaeus brevicornis*.

Keywords: lead, cadmium, crustaceans, inductively coupled plasma – mass spectrometer.

1. Introduction

Trace metals are introduced into the aquatic ecosystems in a number of ways. These chemicals accumulate in the tissues of aquatic organisms at concentrations many times higher than concentrations in water and may be biomagnified in the food chain to levels that cause physiological impairment at higher trophic levels and in human consumers (Raposo et al., 2009). Coastal zones can be considered as the geographic space of interaction between terrestrial and

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marine ecosystems that is of great importance for the survival of a large variety of plants, animals and marine species (Castro et al., 1999). Coastal pollution has been increasing significantly over the recent years and found expanding environmental problems in many developing countries. Urban and industrial activities in coastal areas introduce significant amount of trace metals into the marine environment, causing permanent disturbances in marine ecosystems, leading to environmental and ecological degradation and constitute a potential risk to a number of flora and fauna species, including humans, through food chains (Boran, Altınok, 2010).

There is an increasing concern regarding the roles and fates of trace metals in aquatic ecosystem of Indian Sundarbans. Much of this concern arises from the low level of available information on the concentration of these metals within the environment. Hence, it is very important to determine the concentrations of heavy metals in commercial fish and shrimps in order to evaluate the possible risk of human consumption (Cid et al., 2001).

The use of fin and shell fishes as bio-indicators of metal pollution of aquatic environments and suitability for human use from toxicological point has been documented (Amin et al., 2011). Apart from that, the sensitivity of crustaceans to heavy metals is well documented and for all these reasons, the importance of marine shrimp for environmental monitoring studies as bio-indicators of heavy metal pollution has been emphasized by several investigators (Yilmaz, Yilmaz, 2007).

The concentration of trace metals by seafood is a potential problem to man. The Indian Sundarban regions are no exception to this usual trend. The rapid industrialization and urbanization of the city of Kolkata, Howrah and the newly emerging Haldia port cum industrial complex in the maritime state of West Bengal has caused considerable ecological imbalance in the adjacent coastal zone (Mitra, 1998). The present paper aims to highlight the level of selective trace metals (Pb and Cd) in the muscle tissue of three commercially important species of shrimps, namely *Penaeus monodon, Fenneropenaeus indicus* and *Metapenaeus brevicornis* species selected for this study.

2. Materials and methods

Selection of the sampling stations

Two sampling zones were selected each in the western and central sectors of Gangetic delta at the apex of the Bay of Bengal. The western sector of the deltaic lobe receives the snowmelt water of mighty Himalayan glaciers after being regulated through several barrages on the way. The central sector on the other hand, is fully deprived from such supply due to heavy siltation and clogging of the Bidyadhari channel in the late 15th century (Chaudhuri, Choudhury, 1994). The western sector also receives wastes and effluents of complex nature from multifarious industries concentrated mainly in the upstream zone. On this background four sampling stations (two each in western and central sectors) were selected (Table 1) to analyze the concentrations of heavy metals in the muscles of common edible fishes collected during a rapid EIA study from 2nd November to 12th November, 2016 (Figure 1).

Table 1. Selected sampling stations with coordinates and salient features

Stations	Latitude	Longitude	Anthropogenic activities
Sagar island (Station 1)	21° 39' 04" N	88° 01' 47" E	Situated at the confluence of the River Hooghly and the Bay of Bengal on the western sector of Indian Sundarbans. It is noted for pilgrims in 'mahakumbh mela' every year and unplanned tourism.
Kakdwip (Station 2)	21°52′06″ N	88°11′12″E	Located in the western sector of Indian Sundarbans. Noted as a fish landing station; high anthropogenic pressure is observed on it as a result of unplanned tourism, transportation through passenger vessel jetties, extensive repairing and conditioning of boats and fishing vessels.

Gosaba (Station 3)	22° 15' 45" N	88° 39' 46" E	Located in the Matla Riverine stretch in the central sector of Indian Sundarbans. Noted for unorganized fish landing and shrimp culture.
Sajnekhali (Station 4)	22°05'13.4" N	88 ° 46'10.8" E	Located in the central sector of Indian Sundarbans. Noted for its wilderness and mangrove diversity; selected as our control zone.

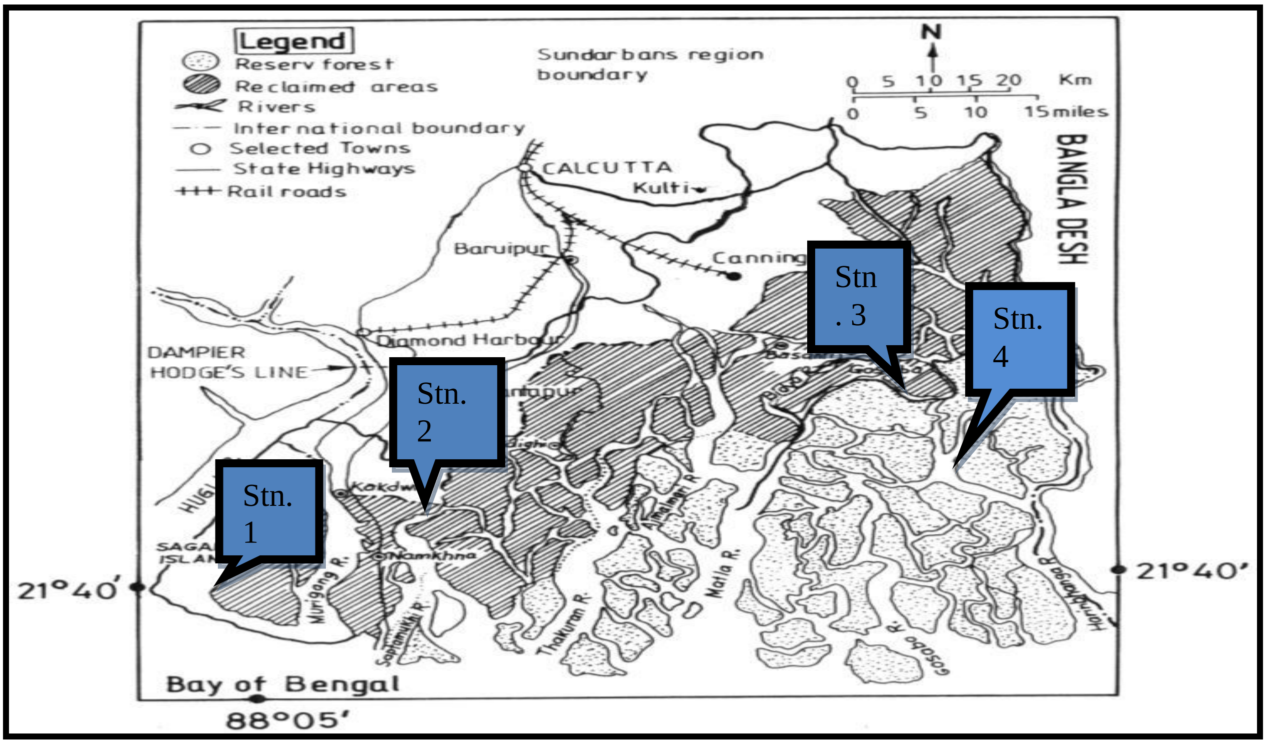

Fig. 1. Map of study area in Indian Sundarbans estuarine region

Collections of specimens

Three species of crustaceans, namely *Penaeus monodon, Fenneropenaeus indicus and Metapenaeus brevicornis* were collected during high tide condition from the selected stations were collected during low tide condition from the selected stations (Table 1). The collected samples were stored in a container, preserved in crushed ice and brought to the laboratory for further analysis. Similar sized specimens of each species were sorted out for analyzing the metal level in the muscle.

Analysis of heavy metals in muscle tissue

Inductively coupled plasma – mass spectrometry (ICP-MS) is now - a - day accepted as a fast, reliable means of multi-elemental analysis for a wide variety of sample types (Date and Gray, 1988). A Perkin-Elmer Sciex ELAN 5000 ICP mass spectrometer was used for the present analysis. A standard torch for this instrument was used with an outer argon gas flow rate of 15 L/min and an intermediate gas flow of 0.9 L/min. The applied power was 1.0 kW. The ion settings were standard settings recommended, when a conventional nebulizer/spray is used with a liquid sample uptake rate of 1.0 mL/min. A Moulinex Super Crousty microwave oven of 2450 MHz frequency magnetron and 1100 W maximum power Polytetrafluoroethylene (PTFE) reactor of 115 ml volume, 1 cm wall thickness with hermetic screw caps, were used for the digestion of the muscle samples of the shellfish. All reagents used were of high purity available and of analytical reagent grade. High purity water was obtained with a Barnstead Nanopure II water-purification system. All glasswares were soaked in 10% (v/v) nitric acid for 24 h and washed with deionised water prior to use.

The analyses were carried out on composite samples of 20 specimens of each species having uniform size. This is a measure to reduce possible variations in metal concentrations due to size and age. 20 mg composite muscle samples from 10 individuals of each species of shell fishes were weighed and successively treated with 4 ml aqua regia, 1.5 mL HF and 3 ml H_2O_2 in a hermetically sealed PIFE reactor, inside a microwave oven, at power levels between 330-550 W, for 12 min to obtain a clear solution. After digestion, 4 ml H_2BO_3 was added and kept in a hot water bath for 10 min, diluted with distilled water to make up the volume to 50 ml. Taking distilled water in place of muscle samples and following all the treatment steps described above the blank process was prepared. The final volume was made up to 50 ml. Finally, the samples and process blank solutions were analysed by ICP-MS. All analyses were done in triplicate and the results were expressed with standard deviation.

Statistical analysis

Analysis of variance (ANOVA) was performed to assess whether heavy metal concentrations varied significantly between sites. Possibilities less than 0.01 ($p < 0.01$) were considered statistically significant. All statistical calculations were performed with SPSS 14.0 for Windows.

3. Result

The accuracy and precision of our results were checked by analyzing standard reference material (SRM, DORM-2). The results indicated good agreement between the certified and the analytical values (Table 2). Number of sample, scientific name, common name and feeding habits of each sample are summarized in Table 3.

Table 2. Concentrations of metals found in Standard Reference Material DORM-2 (dogfish muscle) from the National Research Council, Canada (all data as means ± standard errors, in ppm dry wt.)

Value	Pb	Cd
Certified	0.065	0.043
SE	0.009	0.005
Observed*	0.060	0.040
SE	0.006	0.006
Recovery (%)	92.3	93.0

Table 3. Number of samples and its common name and feeding habit of studied shrimps

Species	Common name	Feeding habit	Number
Penaeus monodon	Black tiger shrimp	Crustaceans, fishes, molluscs and polychaetes	10
Fenneropenaeus indicus	Indian white shrimp	Diatoms, copepoda, ostracods, amphipods, small crustaceans, molluscan larvae, polychaetes and detritus	10
Metapenaeus brevicornis	Yellow shrimp	Vegetable matter, small crustacea, echiurid setae, large crustacea, remains of fishes, polychaeta and Sand grains	10

In this study, the concentrations of Pb in the crustacean muscle were found to range from 4.86 ppm dry wt. (in Sajnekhali) to 9.22 ppm dry wt. (in Sagar island) in case of *Penaeus monodon.* In case of *Fenneropennaeus indicus,* it ranged from 5.81 ppm dry wt. (in Sajnekhali) to 12.8 ppm dry wt. (in Sagar island) whereas, in case of *Metapennaeus brevicornis,* it ranged from 1.02 ppm dry wt. (in Sajnekhali) to 5.87 ppm dry wt. (in Sagar island) (Figure 2).

The concentrations of Pb in the crustacean muscle were found to range from 0.56 ppm dry wt. (in Sajnekhali) to 1.89 ppm dry wt. (in Sagar island) in case of *Penaeus monodon*. In case of *Fenneropennaeus indicus,* it ranged from 0.72 ppm dry wt. (in Sajnekhali) to 3.84 ppm dry wt. (in Sagar island) whereas, in case of *Metapennaeus brevicornis,* it ranged from 0.21 ppm dry wt. (in Sajnekhali) to 2.17 ppm dry wt. (in Sagar island) (Figure 3).

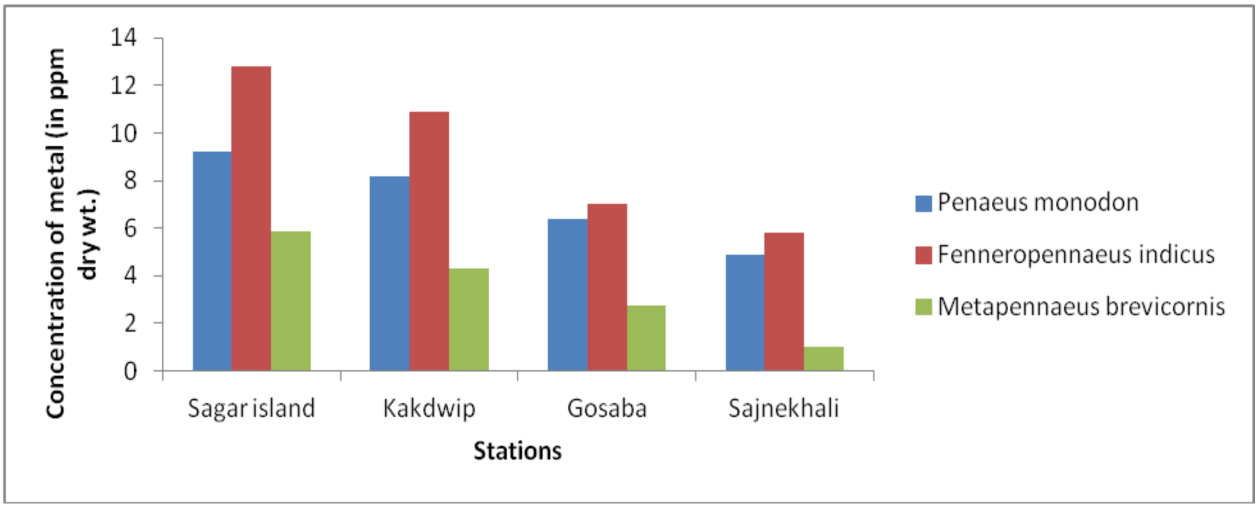

Fig. 2. Concentration of Pb in crustacean muscle in the selected stations for the study period

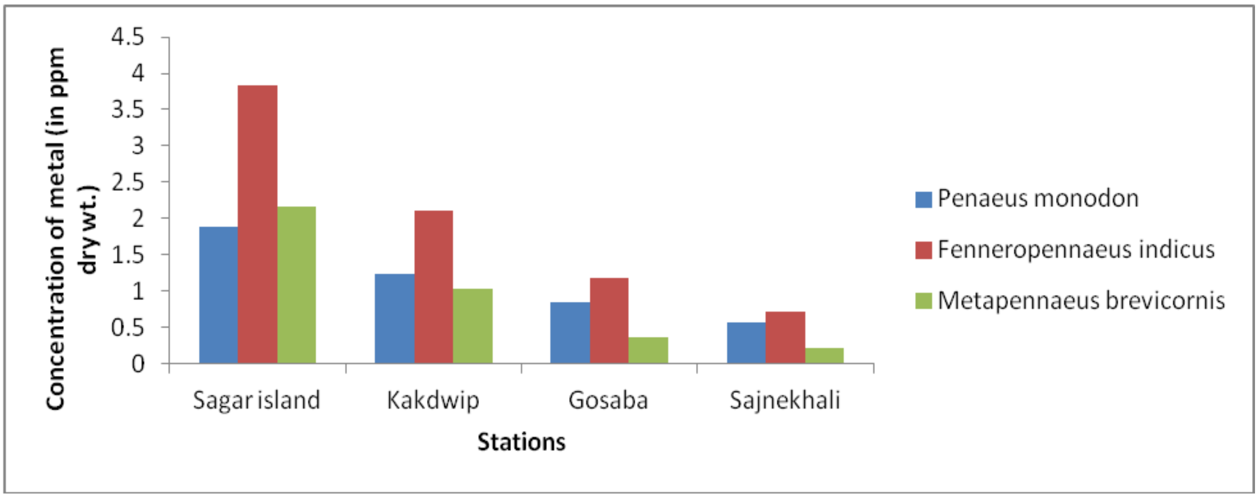

Fig. 3. Concentration of Cd in crustacean muscle in the selected stations for the study period

4. Discussion

Trace element concentrations varied markedly among species. These variations are presumably due to individual samples being of different size categories, from different ecological niches and from different trophic levels. Possibly, species also have variable metabolic requirements for specific trace element (Soegianto et al., 2008). The western part of the Gangetic delta is connected to Himalayan glacier through Bhagirathi River. Researchers pointed out that the glaciers in the Himalayan range are melting at the rate of 23 m/yr (Hasnain, 2002). This along with Farraka discharge has resulted in gradual freshening of the system, which has role in elevation of dissolved metal level in the system by way lowering of pH. The presence of chain of factories and industries along the bank of Hooghly estuary is another major cause of increased metal level in the aquatic phase of Hooghly estuary that have been reflected in the shellfish muscles of stations 1 and 2.

Pb and Cd are non-essential element for most of the living organisms. Pb is a neurotoxin that causes behavioral deficits in aquatic organisms and decreases in survival, growth rates and metabolism (Burger et al., 2002). There is often little accumulation of Pb in marine and freshwater species. Consequently lead is not a threat to fisheries resources except at extreme pollution (Clark, 1997). The most toxic of the heavy metals is Pb, which finds its way in coastal waters through the discharge of industrial waste waters, such as from painting, dyeing, battery manufacturing units and oil refineries *etc*. Antifouling paints used to prevent growth of marine organisms at the bottom of the boats and trawlers also contain lead as an important component. These paints are designed to constantly leach toxic metals into the water to kill organisms that may attach to bottom of the boats, which ultimately is transported to the sediment and aquatic compartments. When compared with the recommended value of World health Organization (WHO, 1989) in context to consumption of seafood (0.5 ppm for Pb), the concentrations in all the shrimp species in all the stations were found much higher than the prescribed limit. Cadmium is regarded as a priority pollutant because of its toxicity to organisms in the aquatic environment (Saddiq, 1992). When excess Cd is absorbed through sea food it tends to accumulate in the liver and in the kidneys of the human body (Mol et al., 2010). The main sources of Cd in the present geographical location are electroplating and an important metal with many industrial applications. In addition to this, Cd is a by-product of Zn and Pb mining and smelting (Mitra, 1998). Very small amount of Cd was recorded in the shrimp muscle from stations 4 that is located almost in industry-free zone surrounded by mangrove vegetation. For Cd, the WHO permissible limit is 0.5 ppm dry wt. for seafood (WHO, 1989). In all the stations and for the entire selected crustacean species, the concentration of Cd were found higher than the prescribed limit; the only exception was Sajnekhali (Station 4) for *Metapenaeus brevicornis*.

The findings of other studies are summarized in Table 4, and are compared with the concentrations reported in this study and elsewhere in the world. In the present study, the pattern of trace metal concentration of five shrimp species were found as order Pb> Cd. Comparing the present data with guidelines and limits (Table 5), it can be seen that most of metal concentrations found in the tissues of aquatic animals proved to be below (excluding Pb) the tolerance levels for human consumption.

Table 4. A comparison of heavy metals concentrations (ppm in dry weight) in crustaceans collected from different parts of the world

Species	Location	Pb (in ppm dry wt.)	Cd (in ppm dry wt.)	References
Fenneropennaeus indicus	Egypt	0.03-0.14	0.04-1.47	Ahdy *et al.*, 2007
Metapenaeus brevicornis	Thane basin, India	0.01-0.02	0.1-0.4	Krishnamurti and Nair, 1998
Penaeus monodon	Bangladesh	0.8-1.3	0.2-0.4	Hossain and Khan, 2001
Fenneropennaeus indicus	Thane basin, India	0.02-0.09	0.04-0.1	Krishnamurti and Nair, 1998
Penaeus monodon	Gulf of Fonseca	0.035-0.5	0.002-0.03	Carbonell *et al.* 1998
Penaeus monodon	Malaysia	BDL	0.002	Ismail et al., 1995
Metapenaeus brevicornis	Uram coast, India	0.4457-2.6529	0.001-0.231	Meshram et al., 2014
Penaeus monodon	Malad area, India	0.213	0.211	Zodape, 2013
Metapenaeus brevicornis	Malad area, India	BDL	0.110	Zodape, 2013

Penaeus monodon	Indian Sundarbans	4.86-9.22	0.56-1.89	In this study
Fenneropennaeus indicus	Indian Sundarbans	5.81-12.8	0.72-3.84	
Metapenaeus brevicornis	Indian Sundarbans	1.02-5.87	0.21-2.17	

Table 5. Maximum permitted concentration of metals in crustacean (*Penaeus monodon, Fenneropennaeus indicus* and *Metapenaeus brevicornis)* as per WHO, 1989

Trace metal	Prescribed limit (in ppm dry wt.)
Pb	0.5
Cd	0.5

Significant variations were observed ($p < 0.01$) in the trace metal concentrations between species. This may be due to variations in potentiality of species towards bioaccumulation. Variations of trace metal concentration between stations may be the effect of dynamic nature of physic-chemical variables in the study area (Table 6). Among the three crustacean species studied the trace metal accumulation followed the general order as: *Fenneropennaeus indicus* > *Penaeus monodon* > *Metapenaeus brevicornis* and among the stations selected for this study the order of metal accumulation was: Station 1 > Station 2 > Station 3 > Station 4.

Table 6. ANOVA results showing spatio-temporal variations between crustacean species and stations

Factors	Variables	F_{cal}	F_{crit}
Muscle Pb	Between species	52.39881	5.143253
	Between stations	27.83714	4.757063
Muscle Cd	Between species	70.18088	5.143253
	Between stations	16.0561	4.757063

5. Conclusion

The shrimp species *Penaeus monodon*, *Penaeus indicus* and *Metapennaeus brevicornis* are commonly available in the mangrove dominated Indian Sundarbans region, at the apex of Bay of Bengal. The knowledge of heavy metal concentrations in crustaceans are very important with respect to nature management, human consumption of these species and to determine the most useful biomonitor species and the most polluted area. Information on the distribution pattern of toxic heavy metal pollutants in aquatic environment becomes important so as to know the accumulation of such pollutants in the organisms and final transfer to man through sea foods. The International official regulatory agencies have set limits for heavy metal concentrations above which the fish is considered unsuitable for human consumption. However in the Indian sub-continent there is no safety level of heavy metals in fish and shrimp tissues. The present zone of investigation situated in and around Indian Sundarbans, a world Heritage Site, demands regular monitoring of metal status for effective management and conservation of this famous mangrove gene pool. The present study is important not only from the human health point of view, but it also presents a comparative account of heavy metals in edible shellfishes from two different sectors of Gangetic delta that are physico-chemically different. The high concentrations of heavy metals in commercially important crustaceans sampled from Nayachar island (station 1) is a cause of concern, and requires regular monitoring of water quality around the point sources present opposite to the western bank of the island, in combination with the fact that shrimp consumption is the main source of heavy metal intake in people not occupationally exposed, amplifies the need for preventive measures to safeguard public health.

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On Localization of Ancient Bearers of Y-DNA R1a Haplotype in Eastern Europe Neolitic Cultures. Part III. New Findings Support the Comb Ware («Pontic Impresso») Version

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Abstract

The work considers the problems of genetics, archeology, and anthropology connected with problem of localization of R1a* Y-DNA haplotype bearers in Mesolithic and Neolithic **Pre-Corded Ware** archaeological sites. Based on the analysis of findings of the 2017 year (described in other works) this paper supports and finds new arguments that the areas of Comb Ware cultures of Eastern Europe could be the possible areas of archaic Y-DNA R1a1 subclades dispersion in the pre-Copper Age period. This paper deals with new haplogroup findings in Eastern Europe and Central Asia. Also the findings of H2a2 in Mongolia are considered as the support of our hypothesis. The link between archaic R1 subclades distribution and Uralic and Altaic language dispersal is developed. We identify two mega-areas of different Neolithic traditions: the one R1b-preM73 dominated, and the second one of «Pontic Impresso» connected with R1a bearers. It is also shown that both groups could move to Altai in the Neolithic times.

Keywords: Y-DNA haplotype, R1a1, R1b1-preM73, Mesolithic, Baltics, Serteya, paleogenetics, paleolinguistics, subclades.

1. Introduction

In our previous works (Semenov, Bulat, 2016, Semenov, Bulat, 2016a) we associate the Neolithic distribution of pre-Corded Ware R1a1 bearers with Comb Pottery area from Pontic Region to the Baikal Lake. Also we noticed, that according to F. Kortlandt's framework, that process could be the initial stage of the dispersal of Proto-Indo-Uralic and Altaic super families. The new genetic findings released in 2017 support this view and give the future directions of study. The new findings in the Ukraine and Estonia supports the hypothesis about the R1a1-M459 dispersal along with the Comb Ware cultures trajectories. Besides, another layer of the wide dispersal of R1b1-pre M73 is identified, which precedes that of R1a1 by time. The latter can be identified with the waves of population movement which affected the Caspian region. The hints on connection between dispersals of haplogroups and those of ceramic styles are being found explicitly.

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2. Materials and Methods

The main materials for the research are data from paleogenetic samples described in other grouped in Table 1.

Table 1. The newest data on paleogenetic samples of Eurasia

Sample	Y-DNA	MtDNA	Source
R1a1			
Dnieper-Donets, Vovnigi 2, Ukraine_N1 4519-4343 BC	R1a1-M459	U4	Pinhasi, 2017, Genetiker
Estonian Comb Ware, Kudrukula 3 3900–1800 BC	R1a5-YP1272 (refined to R1a1-YP1335)	U2e1	Willerslev, 2017, Genetiker
R1b1			
Zvejnieki, Latvia_HG2 5841–5636 BC	pre-R1b1-M73 /M478	U2e1	Pinhasi, 2017, Genetiker
Zvejnieki, Latvia_HG3 5302–4852 BC	pre-R1b1-M73 /M478	U5a2d	Pinhasi, 2017, Genetiker
Zvejnieki, Latvia_MN1 4251–3976 BC	R1b-P297	U4a1	Pinhasi, 2017, Genetiker
Bol'shemysskaya, IV mil. BC	R1b1-P297	No data	Hollard, 2014

The main method is the study of the correlation between ancient haplogroup distribution, archaeological cultures, and ceramic similarities.

3. Discussion and Results

Firstly, the new R1a's were found in Estonia and Ukraine, in the layers which fit the area of Comb Ceramics. The Estonian sample belongs to R1a1-YP1335 (Willerslev, 2017: 8; Genetiker), Dnieper-Donets belongs to R1a1-M459* (Pinhasi, 2017, Genetiker). So we see the presence of another archaic R1a's in Comb Area and in the Pontic Area as we predicted in (Semenov, Bulat, 2016).

Secondly, the mitochondrial haplogroup H2a2, typical for European peoples, was found in the Neolithic Mongolia (Rogers, 2016: 174), what coincides with the migration of R1a1 bearers to the Lake Baikal in the Neolithic times, discussed in (Semenov, Bulat, 2016a). Both R1a1-M17 and H2 were reported in Serteya (West Dvina), in the Pontic area (Smyadovo, Vinogradnoe, H2 only), and in Khvalynsk in the Neolithic-Eneolithic times. So, H2a2 in Asia supports the version of Western roots of R1a in Kitoi culture.

Thirdly, the most important new result was the analysis of the situation in Neolithic Baltics. Last year new interesting data, concerning the population of the Neolithic Narva culture, and Latvian Neolithic in general were released. The latter culture spread within the Baltic States down to the Neva and dated back to VI-III mil. BC (Zinkevičius, 2007). Earlier it was found that representatives of the Narva culture, who lived about 4200 BC had mitochondrial haplogroup U5b (Bramanti, 2009). The new findings are more impressive: Y-haplogroup R1b1 was found in representatives of the Narva culture from Zvejnieki burial ground (Latvia), who lived about 5600 BC till 3900 BC. Also, two I2a1 were found in Spiginas1 and Kretuonas 1B sites (Mittnik, 2017). In the paper (Pinhasi, 2017) it is mentioned that earliest pottery in Latvian sites started around 5400 BC.

It should be mentioned, that two examples of R1b1 found in Neolithic Baltics can be attributed to various subclades (Genetiker) between root R1b1-M297 and R1b1-M73/M478. To this branch also belongs an archaic R1b1 in Middle Volga Culture (Lebyazhinka-IV) (R1b Tree). So, we then suppose, that the ceramics of both cultures could belong to **Azov-Caspian** province and then in the Baltic area could happen the transition to «Pontic impresso» (Comb Ware) which we discussed in previous works (Semenov, Bulat, 2016).

This transition was described by A.M. Miklyaev on the example of nearby cultures of Near Dvina. A.M. Miklyaev defines the evolution of the Serteya ware the following way: «*Sertey culture, encompassing a-c phases of ware development, is the early Neolithic culture. A-phase refers to the pieces of thick-walled, mitre-shaped vessels, manufactured by linear method 'with overlap'. The lines werelinked after the drying and their joints were ironed by comb-like molding tool for the fail-safe joint. Organic matters and shells served as dough plastificator; dough kneads well. The surfaces of finished vessels were coated with the thin layer of clay, and then ornamented in the form of geometric patterns, made in retreat-stroke or (less frequently) only in stroke manner. Vessels were not burnt, just dried. The idea of clay ware, as judged by the motifs and methods of ornamentation (Smirnov, 1989), was probably brought from Azov-Caspian cultural province, but this hypothesis cannot be proved by firm facts yet. But it is possible that Serteya culture was a part of a large early-Neolithic community, stretching from the south of the Russian plane to the Valdai at least*» and further: «*During the next stage, the form of vessels became close to caldron-shape. The ornaments contained more compositions, made by* ***comblike molding tool*** *and the pits and notches, which appeared for the first time. As a rule, the ornament was located in the upper half of vessels and can be called* ***stroke-comb ware****. The range of analogues for this phase is narrower – ware of Upper-Dnieper culture (Artemenko, 1954; Kalechits, 1987) and from the Lithuania territory (Rimantane, 1966 and 1973). It can point to the separation of local groups inside the above-mentioned community. In this particular case the group, located in the interfluve of the Dvina and the Loval rivers, the Upper Dniepr and Lithiania should be mentioned. It is possible that the connection of this group with the Upper Volga Region and the left-bank Ukraine becomes looser*» (Miklyaev, 1992).

Rudnya culture, following immediately after the Serteya culture in the Lovat Region, demonstrates the influence from the Baltics. Thus, if a-phase (Serteya culture) shows the influence of Azov-Caspian Neolithic tradition, b-phase – the influence of Upper-Dnieper cultural community, c-phase – the narrow group of analogues to the Neolithic Age of Lithuania. The influence of the Baltics on the Rudnya culture phase enhances very hard and it can be considered as the evidence of the direct contacts (including migration ones) of Serteya (Uper Dvina) and Baltics: «*Analogies of d-phase ware can be seen in the early-Neolithic monuments of Lithuania (Loze, 1983 and Zagorskis, 1973) and Estonia (Yanits, 1934). Flint industry increases new forms of tools, which analogues are found in the Baltic States*» (Miklyaev, 1992).

So, we see that R1b in Baltics can be inherent to the influence of Azov-Caspian area, and R1a1 in Estonia belongs to the Comb Ware which was explicitly found in Dnieper-Donetsk culture (Genetiker) and in Serteya.

Vovnigi in the Dnieper Area, where R1a1 was detected, is the site, which refers both to Dnieper-Donetsk community and to Mariupol ethnocultural community. Antropological and odonthological analysis showed its European affinities. «*The group from Vovnigi-2 burial ground has a composition slightly different from the other Ukrainian ones. First of all, there is one case of prominent spade-shaped upper incisors. Secondly, its major difference from the others is much more prominent archaism of dentition structure. Finger-shaped crests of the upper incisors and canines, additional distal crest of the upper canins, several cases of hypercone hypertrophic odontogenesis and the posterior fossa of the first upper molars were discovered in this series. The lower premolars had additional mesio cusp, the lower molars had additional central one. It brings the group, having left Vovnigi-2 burial ground and the upper Paleolithic populations of Europe together*» (Zubova, 2016: 147). So far as the stable character of population at the boundary of the Middle and Lower Dnieper Region is mentioned, we can suggest the role of the preceding Bug-Dniester culture in the formation of the population of Vovnigi-2 type. It is another argument for D.L. Gaskevych's hypothesis, who considers Samchinskaya ware of VII-VI millenia BC, as one of the branches of Cardial ware («Pontic Impresso») and the one, preceding the proper Comb ware (Gaskevych, 2010).

So, we see that in the Eastern Europe the Azov-Caspian Influence which can be preliminary associated with the dispersal of R1b-pre M73, is replaced with «Pontic Impresso» (Comb Ware) which is possibly associated with R1a1. The diagram 3 below shows the future hints of possibility (affinity of ceramic styles and Y-DNA haplogroups).

These results can have the possible connections for the history of the Neolithic Central Asia.

Firstly, one of typed Lativans has K1b2 mitochondrial DNA (Gyvakarai1 [9]). The same was reported in Botai culture (Kazakh DNA)]. As it was mentioned in (Zakharov, 2010), Balakhna Pit-Comb area which succeeds Upper Volga culture, and the latter could be connected with Narva and Middle Volga cultures (both with R1b-preM73). So, Botai can be connected with Azov-Caspian province more precisely and have R1b-preM73 bearers. Nevertheless, the first Y DNA data from Botai showed the East Asian O2 Y-haplogroup, which is present in Japanese, Korean, and Tungusian populations (Kazakh DNA). This coincides with the fact of the archeologically attested infiltration of the bearers of the eastern ceramic tradition to the center of the steppe (Semenov, Bulat, 2015), and the mixed character of the Botai.

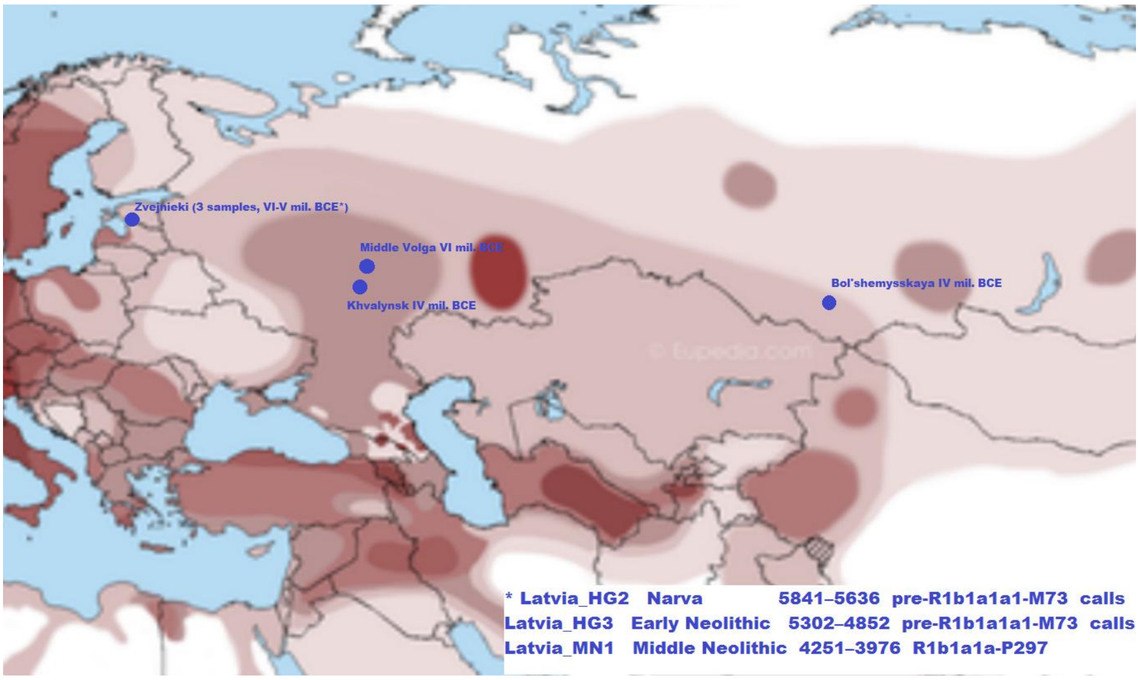

Diagram 1. R1b distribution in Eurasia (www.eupedia.com) with old findings of R1b-preM73.

Another Asian R1b1-P297 was detected in pre-Afanasievo Altai in Bolshemysskaya culture, namely site Tytkesten-VI. In (Kiryushin, 2014: 138) in the mentioned work it is supported the opinion that Bolshemysskaya culture was characterized by the penetration of people from Kazakhstan and Central Asia to Altai. The latter are obviously connected with Caspian region. In is shown than «*Tytkesten-2 (*near Tytkesten-6- authors*) findings fix different elements of material culture, which reflects the penetration to Altai the populations from Central Asia and Kazakhstan. The most probable we see the small group of migrants, who get in touch with local population. In the latter and final Neolithic (first half and middle of IV mil. BC) the penetration became more wide, and this is fixed in the increase in Caucasoid component in the anthropological type of mountain and forest-steppe Altai*». The findings of (Kiryushin, 2014: 138) also show the Comb character of the ceramics, and the resemblance of and Pontic pottery is striking (Diagram 2). So the replacement of R1b-preM73 to R1a1-dominated population in the Eastern Europe, the presence of Comb Ware style in Baikal sites (Semenov, Bulat, 2016a) and the Altai, R1a1 in the Neolithic Baikal, R1b1 in the Neolithic Altai show that the migration from the West was made by mixed R1a1 and R1b1-dominant population. In the Indo-Uralic-Altaic framework, proposed in (Semenov, Bulat, 2016a), we can see the hint that R1b-preM73 earlier expansion reflects the Altaic language dispersal (R1b-preM73 is present in many Altaic language populations), and consequent archaic R1a1 (R1a1-M459) expansion reflects the Indo-Uralic one, according to F. Kortlandt. Though we earlier propose that R haplogroup can belong initially to the bearers of Sino-Caucasic language, the presence of U mtDNA subclades (namely, U5a) in almost all mentioned burials, can lead to conclusion that the languages had become Nostratic-like according to our model. The latter states that groups of male R1 bearers marry the bearers of U5 and possibly other U's as mitochondrial haplogorups, what lead to the language transformation.

Definitely, we see that in Asia Comb ware ceramics was present and Asian Comb ware cultures possibly had the representative of previous Azov-Caspian wave (R1a1 in Baikal and R1b1-M297 in Bolshemysskaya).

Presence R1a1 and H2 in Neolithic Central Asia and striking resemblance of vessels show that Pontic influences (according to D.L. Gaskevych) could enter the Altai area or they have common center somewhere in Eurasia.

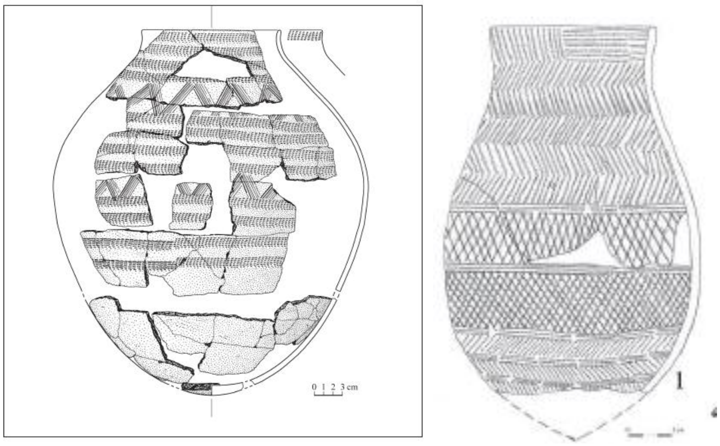

Diagram 2. The vessel from the «Pontic Impresso» (Comb Ware prototype (Gaskevych, 2010) – left, the vessel from pre-Afanasievo Altai (Kiryushin, 2014) – right

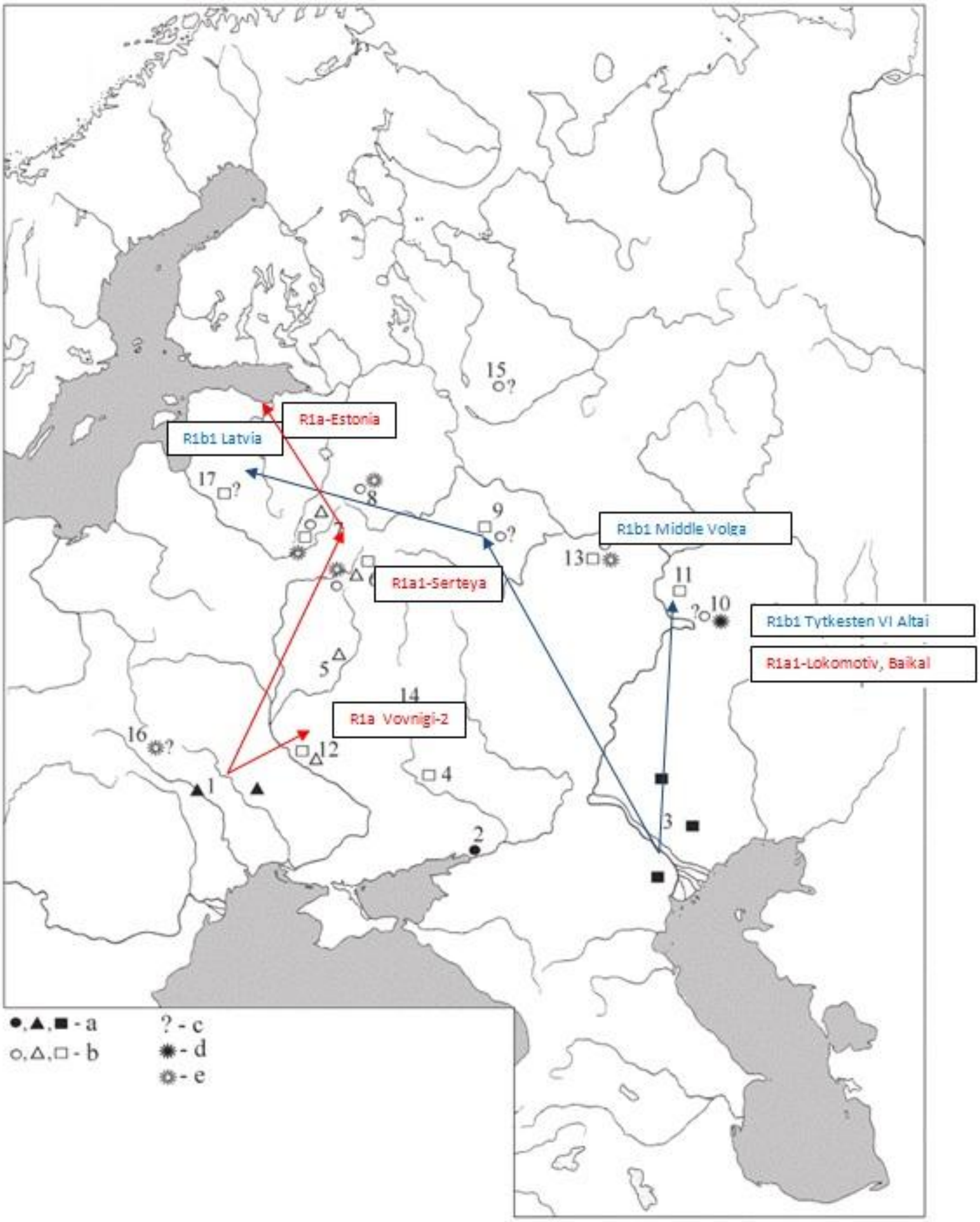

Fig. 1. Distribution of the Early Neolithic pottery traditions, cultures and sites in Eastern Europe (*a* — primary centers; *b* — sites with pottery traditions influenced by the primary centers; *c* — influence is probable; *d* — Yelshanian pottery (*10*); *e* — Yelshanoid pottery; *1* — Bug-Dniester culture; *2* — Rakushechny Yar; *3* — Low Volga culture; *4* — Middle Don culture; *5* — Desninskaya culture; *6* — Upper Dnieper culture; *7* — Serteyskaya culture; *8* — Valdayskaya culture; *9* — Upper Volga culture; *11* — Middle Volga culture; *12* — Dnieper-Donets culture; *13* — sites of the Sura-Moksha basin; *14* — Karamyshevo 5; *15* — Berezovaya Slobodka II–III, VI; *16* — Gora Strumel'; *17* — Zvidze)

Diagram 3. The grouping of the Neolithic ware (Mazurkevich, 2013) and the possible correlation with Y-DNA haplogroups (added by the authors).

4. Conclusions

In the light of all the above-stated, we can make the final conclusions:

1. The influence of the Middle-Volga Region can be the first significant Neolithic influence in the Baltic region (eventually from Volga-Ural Region), what can result in genetic continuity between Latvia and Volga in Neolithic times.

2. We can suppose the existence of some kind of a belt of the expansion of male haplogroup R1b1-preM73 from the Neman to the Caspian Sea and farther to the east.

3. Finally, the emergence of R1a1 in the region can be connected with the later influence of the Lower Dnieper «Pontic Impresso» cultures (via Dnieper-Donets culture and preceding).

4. The dispersion of the Comb Ceramics could go along with R1a1 and also R1b-preM73 Y-haplogroup bearers (maybe in minor quantity and share).

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