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Bioaccumulation indexes of metals in blue crab inhabiting specially protected area Koycegiz Lagoon (Turkey)

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ABSTRACT

The paper presents original results concerning individual total metal load (IMBI) values, metal pollution index (MPI) and concentrations of 11 metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn, As, Hg and Se) in blue crab (*Callinectes sapidus* Rathbun 1896) inhabiting Köyceðiz Lagoon System Turkey. Total of 60 blue crab samples were caught and investigated monthly between June 2009 and May 2010. Concentrations of trace metals in tissues were measured by ICP-AES. Concentration of Cr, Fe, As and Hg were found higher in spring than other season while Cd, Zn, As, Hg and Se showed highest concentration in hepatopancreas. IMBI values in all tissues were higher on spring and summer. On the other hand IMBI values calculated were higher in shell than other tissues. Result of high IMBI values on spring can explain due to the fact that increasing MPI value of Cd, Pb, Fe and Zn on spring while high IMBI values on summer affected by high MPI value of Cd, Pb and Mn. Cd accumulation on autumn and spring also Pb accumulation on every season in blue crab were found higher than the European Units limits for human consumption in edible parts of the crab samples and posed a risk for human health.

Key words: *Callinectes sapidus*, Heavy metal, IMBI, Koycegiz, MPI

Hundreds of pollutants are discharged into the environment every day. Accumulation of heavy metals in aquatic ecosystems is of global importance. Of these, heavy metals are regarded as serious pollutants of the aquatic environment because of their environmental persistence and tendency to be concentrated in aquatic organisms (Harte *et al*. 1991, Schüürmann and Markert 1998). Metals generally enter the aquatic environment through atmospheric deposition, erosion of geological matrix or due to anthropogenic activities caused by industrial effluents, domestic sewage and mining wastes (Gumgum 1994, Alam *et al*. 2002, Forstner and Wittmann 1979).

Macrobenthos play a major role in material flow of the lagoon systems. As a primary consumer, they accumulate more essential elements, such as zinc and iron, than the primary producers do. Trace metals are toxic at relatively low concentrations. These are taken up and accumulated by aquatic invertebrates to concentrations much higher on a weight basis than concentrations in the surrounding seawater (Rainbow 1988, Depledge and Rainbow 1990).

Sources of variation in trace metal concentrations include variability between and within species (e.g., sex, life history stage, length), and spatial and temporal variability (Zauke and Petri, 1993).

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Koycegiz Lagoon System, declared as a Special Protection Area in 1988 (Bayari *et al.* 1995) is located in south-western of Turkey. The area is composed of terrestrial structures of various qualities around Köyceðiz Subsidence Lake. The lagoon is under pollution pressure of agricultural run-off, untreated urban waste and tourist-boat traffic (Yorulmaz *et al*. 2008).

Crabs of the genus *Callinectes* (Crustacea: Portunidae) are abundant in Koycegiz Lagoon System. These are the basis of a significant commercial harvest and recreational fishery. The contaminated crab from this area may become a public health concern. Therefore, in order to assess the metal contamination of the aquatic environment of lagoon system, information on elemental concentration in crab species in Koycegiz Lake and Lagoon System becomes of great importance.

The aim of this work was to determine the distribution of heavy metal in blue crab. For this purpose, concentrations of heavy metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn, As, Hg, Se) were measured in tissues (gill, hepatopancreas, shell and muscle) of blue crab and also performed statistical analyzed for condition factor and IMBI values to compare heavy metal accumulation. MPI was used to find IMBI values are affected by which metals.

MATERIALS AND METHODS

Study area: The Koycegiz Lake estuary is a system of vital importance in South-West of Turkey, not only from the physical viewpoint but also because of its ecological significance. This area is among the most attractive tourist sites on the Mediterranean coast of Turkey (Bayari *et al.* 1995). Sampling was conducted every month during 2010 on Koycegiz Lagoon system, locations of which were N. 36° 49' 49'', E. 28° 38' 23''.

Collection of crabs: Crabs were collected every month during June 2009 to May 2010 either using a star trap or a dip net. Approximately 5 crabs were collected during each sampling trip. After each catch, crabs were rapidly placed inside polyethylene bags and transferred to an icebox at approximately 5°C. After 1–2 h, these were placed in a freezer at –80°C.

Digestion and heavy metal determination: Tissues of blue crab from the same station were pooled and all tissues were transferred to an oven set to 120°C to dry. Drying continued until all the wet tissues reached to a constant weight, dry tissue samples were put into digestion flasks and 30% hydrogen peroxide and 70% nitric acid were added. The digestion flasks were then put on a microwave digestion unit to 120°C (gradually increased) until all the materials were dissolved. After digestion the digested samples were diluted with distilled water appropriately in the range of standards which were prepared from stock standard solution of the metals.

Concentrations of all metals in the digestives were determined by spectroscopy.

Data analyses: Kruskal-Wallis and Man-Whitney tests were used to access whether metal concentrations varied significantly between tissues and season, possibilities less than 0.05 ($P<0.05$) were considered statistically significant. Pearson's test also used to correlate metals accumulation with sex and season. All statistical calculations were performed with SPSS 20.0 for Windows. The individual mean (multi-metal) bioaccumulation index (IMBI) was calculated as:

$$IMBI=\frac{\varepsilon_i^n N=IC_i/C_{imax}}{n}$$

With N the total member of metals, C_i the individual metal concentration of heavy metal I, C_{imax} the maximal observed concentration of heavy metal I and $0<IMBI<1$ (Maes *et al.* 2008).

The metal pollution index (MPI) was used, obtained with the equation (Usero *et al*. 1997):

$$MPI = (Cf_i \times Cf_2 \ldots Cf_n)^{1/n}$$

Where, Cf_i,concentration for the metal i in the sample.

RESULTS AND DISCUSSION

Condition factor of blue crab: More than 60 individual of blue crab (37 male and 23 female) were caught. Some average parameters (length of carapace in cm weight of crab in g) and the mean condition factors weight and length of blue crabs were calculated (Table 1).

In this study measuring the length and weight were difficult because blue crab can be broken or can be injured by predator. In addition researchers also can be injured by blue crab. The longest carapace length of blue crab (21.5 cm) was measured on October while the shortest carapace (15 cm) was measured on May. The maximum crab weight was determined on April as 426 g and minimum weight of crab was determined on January as 130 g. The mean condition factor for males was significantly higher in females (Table 1) but condition factor oscillated throughout the sampling year. The condition factor is strongly influenced by the environment factors (exogenous parameters) and by the gonad development, the rate of

Table 1. Condition factors (K) by the carapace length (L) and weight (W) for blue crab caught in Koycegiz Lagoon (mean±SE, n=60).

Sex	N	$[K=(W*100)/CW^3]$	$[K=(L*100)/CL^3]$	Carapace length (cm)	Weight (g)
Male	37	43.460±12.362	4.631±1.413	17.748±1.868	253.271±69.999
Female	23	33.896±14.337	3.311±1.492	18.004±1.273	186.760±67.634
Both	60	39.475±14.012	4.081±1.583	17.855±1.65	225.558±76.314

Table 2. Mean concentrations (µg/g) values of metals in tissues of blue crab in Köyceiz Lagoon (average±SE, n=60)

Tissue	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn	As	Hg	Se
Muscle	0.161 $\pm0.24^{ac}$	1.813 $\pm0.41^{a}$	18.214 $\pm2.60^{a}$	9.615 $\pm3.33^{a}$	6.037 $\pm3.43^{a}$	1.023 $\pm0.15^{a}$	1.208 $\pm0.13^{a}$	43.981 $\pm3.44^{a}$	0.265 $\pm0.12^{a}$	0.090 $\pm0.01^{a}$	0.074 $\pm0.02^{a}$
Gill	0.189 $\pm0.018^{a}$	4.012 $\pm1.03^{b}$	73.495 $\pm11.62^{b}$	173.074 $\pm39.66^{a}$	14.179 $\pm1.63^{b}$	4.523 $\pm0.81^{b}$	2.230 $\pm0.29^{bc}$	25.852 $\pm2.36^{b}$	0.359 $\pm0.14^{a}$	0.111 $\pm0.02^{a}$	0.105 $\pm0.04^{a}$
Hepato-pancreas	0.893 $\pm0.12^{b}$	2.856 $\pm0.74^{ab}$	50.169 $\pm13.04^{c}$	32.961 $\pm21.88^{b}$	6.787 $\pm1.05^{c}$	2.165 $\pm0.24^{c}$	1.386 $\pm0.13^{a}$	55.202 $\pm13.21^{a}$	0.868 $\pm0.22^{a}$	0.126 $\pm0.03^{a}$	0.163 $\pm0.06^{a}$
Crust	0.121 $\pm0.01^{c}$	2.680 $\pm0.45^{cb}$	9.322 $\pm1.60^{d}$	37.946 $\pm7.43^{c}$	106.874 $\pm12.08^{d}$	1.689 $\pm0.26^{d}$	2.125 $\pm0.19^{c}$	16.716 $\pm2.24^{c}$	0.505 $\pm0.15^{a}$	0.057 $\pm0.01^{a}$	BDL

BDL:Below the Detection Limit

feeding and growth and the degree of parasitism (endogenous parameters) and may vary among seasons and populations (Lecren 1951, Rodriguez 1987, Vazzoler 1996, Froese 2006, Pinheiro and Fiscarelli 2009). Significant differences could be detected among the seasons, in male and female the CL (length) and CW (weight) is high in spring, and exhibits a sharp decrease in summer. In the following seasons, there is an increase of the CL and CW again. Results showed that male being larger and heavier than female because males present a mate-guarding behavior during and after copulation, providing protection to the recent post-molt female (Pinheiro and Fransozo 1999). In fact, adult males may have to be stronger to be able of such behavior. On the other hand, females direct a large portion of the energy budget to the eggs production (Kotiaho and Simmons 2003).

Metal accumulations in blue crab tissue: Mean concentrations of Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn, Hg, As and Se in tissues of Blue crab from the Koycegiz Lagoon is shown in Table 2.

According to obtained results, in the muscle and hepatopancreas tissues, highest Zn accumulation were observed with a mean of 43.98 µg/g and 55.20 µg/g respectively. In gill tissue, mean highest accumulation was observed at Fe with 173.07 µg/g while the mean highest accumulation in shell tissue was at Mn with 106.87 µg/g levels.

The accumulation of investigated heavy metals in muscle tissue was in order as;

Zn > Cu > Fe > Mn > Cr > Pb > Ni > As > Cd > Hg > Se.

In gill tissue the order of accumulation of the investigated heavy metals was as;

Fe > Cu > Zn > Mn > Ni > Cr > Pb > As > Cd > Hg >Se.

In hepatopancreas tissue the order of the accumulation of the investigated heavy metals was Zn > Cu > Fe > Mn > Cr > Ni > Pb > Cd > As > Se > Hg while it was, Mn > Fe > Zn > Cu > Cr > Pb > Ni > As > Cd > Hg > Se in shell tissue.

Concentrations in muscle and gill are differed significantly among seasons for Cr, Cu, Fe, Mn, Ni, Pb and Zn ($P < 0.05$). The significant differences were found for Cd, Cu, Mn and Ni ($P<0.05$) between liver and hepatopancreas ($P<0.05$). Significant differences between muscle and hepatopancreas for Zn ($P<0.05$) were not found but in other tissues there are significant differences. The Kruskal-Wallis test showed the significant seasonal differences for Ni ($P<0.05$) in all tissues.

The results confirmed the differences of heavy metal accumulation in the different tissues. The highest metal concentrations were found in hepatopancreas, muscle and gill except for Se and Hg. The data showed that Cd, Cr, Ni, Pb, Zn, As and Hg concentration in hepatopancreas is much higher than other tissues and also many studies (Gey 1983, Kargin 1996, Usero *et al.* 2003, Dural *et al.* 2007) showed that heavy metal accumulation in liver is higher than other tissues.

Türkmen *et al*. (2006) investigation regarding blue crabs of Mediterranean Sea of Turkey revealed that the crabs contain Cd 1.06–2.51 µg/g, Co 2.53–3.17 µg/g, Cr 2.82–6.56 µg/g, Cu 3.88–9.39 µg/g, Fe 7.25–23.27 µg/g, Mn 1.83–8.48 µg/g, Ni 1.63–4.19 µg/g, Pb 2.67–4.30 µg/g, Zn 6.67–11.5 µg/g. These metal concentrations are relatively high for Cd, Cr, Ni, Pb, but less for Cu and Zn compared to those in the Koycegiz Lagoon System.

Sastre *et al.* (1999) describes variations in concentrations of Cu, Cd, Pb and Hg, between species, sexes, tissues and sites in *C. sapidus* and *C. bocourti* in Puerto Rico. They found Cu concentrations of all tissues of *C. sapidus* (mean 5.912 µg/g wet) and *C. bocourti* (mean, 10.102 µg/g wet) and Cu concentrations in present study much higher than their findings. But Average mercury levels in their study (species, sexes and tissues pooled) ranged from 0.37–0.50 µg/g (wet wt); this metal concentration was much higher than our Hg concentrations.

Yilmaz (2009) measured ometals accumulation (Cd, Cu, Mn, Pb, Zn) of three fish species *(Anguilla anguilla*, *Mugil cephalus*, *Oreochromis niloticus*) caught from the Koycegiz Lagoon between June–2005 and May–2006. Present results about accumulation of metals in gill were high for all metals except Zn than their findings. The gills are uptake site of water-borne ions, where metal concentrations increase especially at the beginning of exposure, before the metal enters other parts of organism (Heath 1987).

High accumulation of Mn in shell was found in April-June. The amount of oxygen in water was determined high due to the rains and circulation of Lake. High value of dissolved oxygen in water increased solubility of manganese and thus get accumulated by organisms more. The lowest levels were detected in the shell, except for Mn.

Seasonal variations of metal in the tissues: Mean concentrations of Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn, Hg, As and Se accumulation on autumn, winter, spring and summer are given Table 3.

Average heavy metal accumulation of blue crab was investigated every season; the highest accumulation of heavy metal for each season was Fe. The maximum accumulation of Fe (118.413µg/g) determined on spring.

Table 3. Mean concentrations (µg/g) of metal accumulation of Blue crab on seasonally (average±SE, n=60)

Metals	Spring	Summer	Autumn	Winter
Cd	0.182±0.02	0.425±0.06	0.638±0.12	0.121±0.02
Cr	4.104±0.78	3.089±1.10	2.301±0.25	1.869±0.32
Cu	33.200±4.76	38.007±5.49	60.528±16.82	19.462±2.86
Fe	118.413±34.31	57.841±22.96	32.585±23.88	44.755±6.99
Mn	45.239±11.21	24.433±4.72	46.410±10.39	17.803±4.14
Ni	1.804±0.59	2.828±0.29	3.349±0.62	1.420±0.25
Pb	1.600±0.16	2.086±0.14	2.245±0.28	1.020±0.17
Zn	34.683±2.71	27.365±2.60	27.039±2.27	52.670±13.62
As	1.564±0.27	0.064±0.02	BDL	0.369±0.11
Hg	0.154±0.01	0.090±0.01	0.060±0.03	0.081±0.02
Se	BDL	0.342±0.07	BDL	BDL

BDL: Below the detection limit.

Lowest Se concentrations were detected in all tissues on every season except summer whereas in summer Se concentration was 0.342 µg/g. Highest total Cd concentration was found in hepatopancreas on autumn while concentration of Cd was determined lowest on other seasons. The highest Pb metal concentration determined on every seasons (spring: 1.600 µg/g, summer: 2.086 µg/g, autumn: 2.245 µg/g, winter: 1.020 µg/g). All metal concentration, analyzed with Man-Whitney test revealed that Cd, Cr, Cu, Fe, Pb, As and Hg levels in winter differed significantly from that in spring. In Se, measurements made in summer differed significantly from all other seasons. The significant differences in all seasons were not found for Mn ($P<0.05$) but found in all tissues. As and Hg exhibited not significant differences in all tissues. But significant differences for Hg were identified among seasons ($P<0.05$). The significant differences in all metals except for Mn were detected between summer and spring ($P<0.05$). Metal concentrations in gill and shell did not differ significantly for As, Hg, Se, Cr ($P<0.05$) but significant differences for As, Hg and Se were identified between summer and spring and also summer and autumn. Man-Whitney test indicated that the differences between summer and autumn were not significant for Pb but significant in other seasons ($P<0.05$). Zinc levels on winter were different from summer, spring and autumn ($P<0.05$). Results obtained in winter differed from spring for Ni with Man-Whitney test.

Spring was different from all seasons be cause of highest concentration of Cr, Fe, As and Hg, and highest Cd, Zn, As, Hg and Se concentration were found in hepatopancreas. Based on total content of accumulated metals, tissues could be generally ordered as hepatopancreas>gill>muscle>shell. Cd, Cu and Ni were mainly accumulated followed by autumn> summer>spring>winter.

Most metals in the physical environment are tightly bound to the sediments and therefore not bio-available (Hall and Pulliam 1995). Blue crabs live on sediments on which they bury from where they mainly feed. The high bioaccumulation level of crab might be related to a number of factors such as, rigorous anthropogenic activities, bioavailability of chemicals in food and water and physico-chemical parameters of the aquatic environment and crabs are less active and their feeding habits, ecological needs, other characteristic behaviors and metabolism different than other organism (Türkmen *et al.* 2006, Pieters and Geuke 1994, Joiris *et al.* 1997, Mason *et al.* 2000).

Metal concentrations according to sex and season: The significant differences were not found for Cd, Cu, Fe, Mn, Ni, Pb, Zn and As, except Se and Hg ($P<0.05$) between male and female tissues. The quantitative analyses of the possible relationships in crab tissues between element pairs were carried out among eleven variables such as Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn, As, Hg, Se in seasonally. In winter the data showed very high levels of correlation ($P<0.05$) with mostly positive values. Significantly positive relationships were found in winter between Fe-Cr, Fe-Cu, Ni-Cr, Ni-Cu, Ni-Fe, As-Mn, Hg-As, Pb-Cd, Pb-Cu. On the other hand, in spring the data showed high levels of correlation ($P<0.05$) with mostly positive values except very few negative values among different pairs of variables. Significantly positive relationships were found in spring between only Cu-Cd, Mn-Cu, Ni-Cu, Ni-Fe, Pb-Mn, while negative relationships were found between Zn-Pb and As-Fe. Other trace metals showed weaker relationships. Additionally in summer, data showed a highly positive correlation among different pairs of variables. Almost all the trace metals have highly positive correlations except Zn-Mn. Finally in the autumn data showed a positive correlation between Cr-Cd, Fe-Cr, Ni-Fe, Zn-Cd, Zn-Mn. However, different organs showed significant correlation in metals bioaccumulation, which may be different in aquatic environment, uptake, sex and age of the crab. This study showed that being male or female does not matter for the accumulation of heavy metals. Bioaccumulation of metals can only take place if the rate of uptake by an organism exceeds the rate of elimination (Specie and Hamelink 1985).

Individual metal bioaccumulation index, metal pollution index and human consumption: IMBI were useful for determining metal accumulations in many articles for European eel *Anguilla anguilla* (Maes *et al.* 2008, Esteve *et al.* 2012, Maes *et al.* 2005). The distribution of total metal load values ranged from 0.00 to 0.30, 0.00 to 0.40, 0.00 to 0.30, 0.00 to 0.38 in muscle, gill, hepatopancreas and shell respectively. Total metal values in all tissues were higher in spring and summer when compared with other seasons. On the other hand IMBI values calculated higher on winter for shell. Usually IMBI values determined on higher in shell than other tissues. We also performed statistical analysis between condition factor and IMBI values to compare heavy metal accumulation relationship. This analysis was performed including male and female and also in all tissues and there was no correlation between accumulation if female and male and also no difference was observed in tissues. This study showed that metal accumulation does not change according to gender and tissues.

IMBI can explain distribution of total metal load seasonally but cannot introduce which metals are affecting these values. For this reason MPI values analyzed by seasonally and Cu (5.78), Fe (6.47) and Pb (9.39) values were found higher in spring while Mn (9.50) value was very high in summer. Cd values for MPI were higher in all seasons except in winter. IMBI values of most of the metals varied notably depending on the MPI values on seasonally. Result of high IMBI values in spring were due to increasing MPI value of Cd, Pb, Fe and Zn in spring while high IMBI values in summer were due to high MPI value of Cd, Pb and Mn. IMBI and MPI value of Cd and Pb are found very high.

Dietary standards and guidelines applicable for heavy metals in crustaceans were summarized by EU (2006). Considering limit value for human consumption of metals is shown in Table 4; 0.50 (µg/g) for Hg, 0.50 (µg/g) for Cd, 0.50 (µg/g) for Pb, metal concentrations in muscle is

Table 4. Considering limit value for human consumption of metals for Crustaceans (μg/g)

	Cadmium (Cd)		Lead (Pb)		Mercury (Hg)	
	EU Max.limit (μg/g)	Periodic Average (μg/g)	EU Max. limit (μg/g)	Periodic Average (μg/g)	EUMax. limit (μg/g)	Periodic Average (μg/g)
Winter	0.50	0.877±0.02	0.50	0.745±0.30	0.50	0.051±0.02
Spring		0.873±0.01		1.206±0.29		0.138±0.04
Summer		0.204±0.01		1.468±0.19		0.139±0.03
Autumn		0.265±0.08		1.413±0.23		0.032±0.02

maximum limit for crustaceans.

This study was carried out to provide information on heavy metal concentrations in *C. sapidus* from SW Anatolia. Cd accumulation in winter and spring also Pb accumulation in every season were higher than limits for crab proposed by EU (2006). According to our results, the examined crabs were associated with enhanced metal content in their muscle and were not safe within the limits for human consumption.

This study is also the first information on IMBI, MPI and concentration of metals in blue crab tissues in Koycegiz Lagoon System. The data showed accumulation of heavy metals (Fe, Cu, Zn and Mn) in crabs to a considerable extent, and relatively more than that reported from other regions in the literature. The study indicated that crabs may be more suitable biomonitors of metal in the surrounding environment than other aquatic organisms.

The lagoon is under pollution pressure of agricultural run-off and untreated urban waste. Heavy tourist-boat traffic on the canals between the lake and the sea causes heavy metal pollution.

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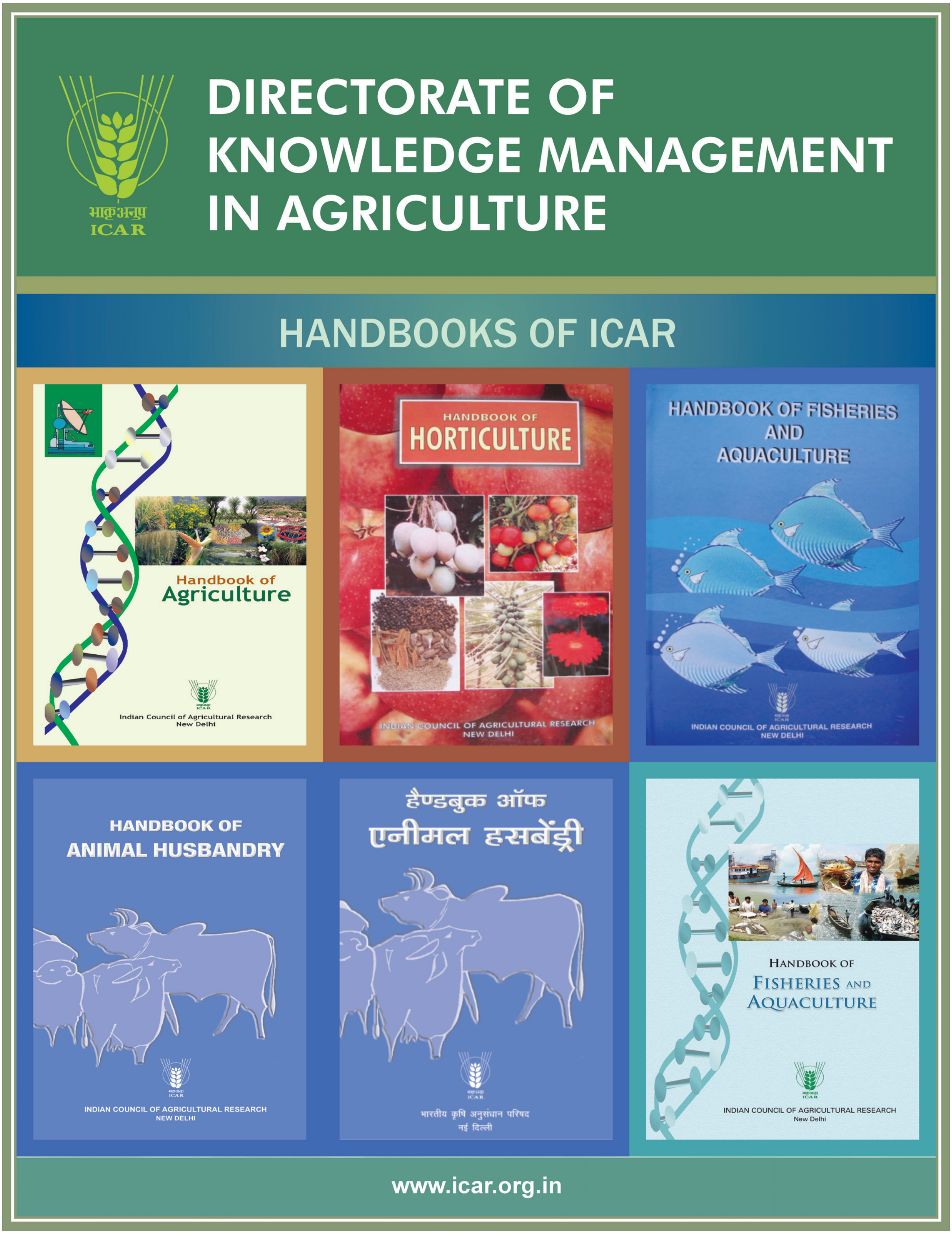
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